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U.S. NATIONAL CANCER INSTITUTE

ANNUAL REPORT of PROGRAM ACTIVITIES.

July 1, 1976 through September 30, 1977

Part VI

DIVISION OF CANCER TREATMENT

U.S NATIONAL CANCER INSTITUTE

ANNUAL REPORT

July 1, 1976 through September 30, 1977

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ANNUAL REPORT

DIVISION OF CANCER TREATMENT

Vincent T. DeVita, Jr., M.D.

Director

The Division of Cancer Treatment is responsible for the development of new treatments for the control and cure of cancer. It is the main component of the National Cancer Program responsible for coordinating cancer treatment within the various elements of the National Cancer Institute, the country and the world. The Division has four major programs, each with a separate and distinct responsibility. These programs encompass the identification of new materials or methods for the treatment of cancer at the very earliest level in rodents; their evaluation for effectiveness in standard tumor systems; the gathering of information on toxicology in preparation of IND's for submission to the Food and Drug Administration for approval to initiate clinical trials in people; the conduct of clinical trials at the earliest level (Phase I); the conduct of disease-specific trials (Phase II); and finally, if therapeutic efficacy is demonstrated, comparative clinical trials against what we now consider standard treatment of cancer (Phase III).

During the past year, the major preclinical drug development activities, previously split between the Drug Research and Development and the Experimental Therapeutics Programs, were consolidated into a single unit, the Developmental Therapeutics Program under the direction of Dr. Vincent Oliverio. Dr. Saul Schepartz, formerly Associate Director for Drug Research and Development, has become Deputy Director of the Division of Cancer Treatment. The Developmental Therapeutics Program, as now constituted, consists of a wide variety of functions, including drug design and development, development and use of systems for drug evaluation, large-scale production, dosage formulation, toxicology, pharmacology, molecular biology, and other related activites representing an integrated preclinical approach to drug development.

The change in the organization of preclinical activities should faciliitate a more rapid and efficient development of new active agents, an objective that represented a significant effort during the past year. A number of other actions were also taken to move drugs more rapidly to the clinic. First, a series of committees were established to review the major classes of analogs and recommend specific drugs for further development in each class, direction of research efforts, etc. Secondly, through a series of meetings reviewing all materials under development, a number of low priority compounds were dropped to allow a greater concentration of effort on those of more interest, while simultaneously a group of high priority compounds were identified as being of special interest. Third, individual advocates were identified to assure maximum speed of development of the high priority compounds. These actions should result in those compounds reaching clinical trial earlier than would normally have been the case.

The flow from the Developmental Therapeutics Program is directly into the Cancer Therapy Evaluation Program headed by Dr. Franco Muggia, the Associate

Director. This Program is responsible for the initiation and monitoring of all Phase I clinical trials conducted under the auspices of the Division. It is responsible through contract for Phase II clinical trials and in some instances for disease-specific Phase III clinical trials such as those in gastrointestinal cancer conducted by the Division's Gastrointestinal Cancer Study Group. In addition, the clinical Cooperative Group program is in this area and a major responsibility of the staff of this program is to monitor the progress of all clinical trials being conducted under the auspices of the National Cancer Institute in the United States, and in the world. In addition, they serve as suppliers of chemotherapeutic agents to investigators throughout the world. They monitor these trials to provide data for the Food and Drug Administration in regard to patient safety.

Two other programs within the Division of Cancer Treatment are primarily concerned with conducting laboratory research and clinical trials in specific problems in cancer of interest to investigators at the NIH.

One is the Baltimore Cancer Research Center. The Baltimore Cancer Research Center has both a clinical and laboratory capacity. The major program in the management of acute leukemia in adults is located at the Baltimore Cancer Research Center, and much work is going on in the pharmacology of the anthracycline antibiotics in this area. The two are inter-related since the disease, acute myelocytic leukemia, is effectively treated with anthracycline antibiotics. The oncology program of the Baltimore Cancer Research Center has recently been reorganized and divided into the Clinical Oncology Branch, the Laboratory of Molecular Biology and the Laboratory of Clinical Biochemistry. The Program is headed by Dr. Michael Walker, who is a neurosurgeon, and one of the responsibilities of the Program is to monitor the nationwide Brain Cancer Task Force exploring the efficacy of the use of combined modalities of treatment in brain cancer.

The remaining program, the Clinical Oncology Program, headed by Dr. John Ziegler as Associate Director, consists of five intramural clinical branches; Surgery, Radiation Therapy, Medicine, Pediatrics, and an additional Medical Oncology Branch located at the Veterans Administration Hospital in Washington, D. C. Each of these Branches identifies specific disease areas to focus their attention on. In conjunction with therapeutic trials in these specific disease areas, investigators in the laboratory explore the mechanism of disease and the biochemistry of carefully controlled clinical situations. It is here that the supportive care programs in the white blood cell procurement and transfusion, platelet physiology and sterile environments have been developed and continue to be explored.

In the past year the Division instituted a newsletter. The purpose of the newsletter is to make new treatment information available to physicians performing clinical trials around the country, to inform clinicians of new scientific advances of potential value in the design of clinical trials, and to alert laboratory scientists to opportunities in the clinics.

After a trial period of publication, a readership survey will be conducted to determine the response to the newsletter. The survey will help to narrow the mailing list to those with an expressed interest in the newsletter and to determine more specifically which areas of research are most significant for the recipients.

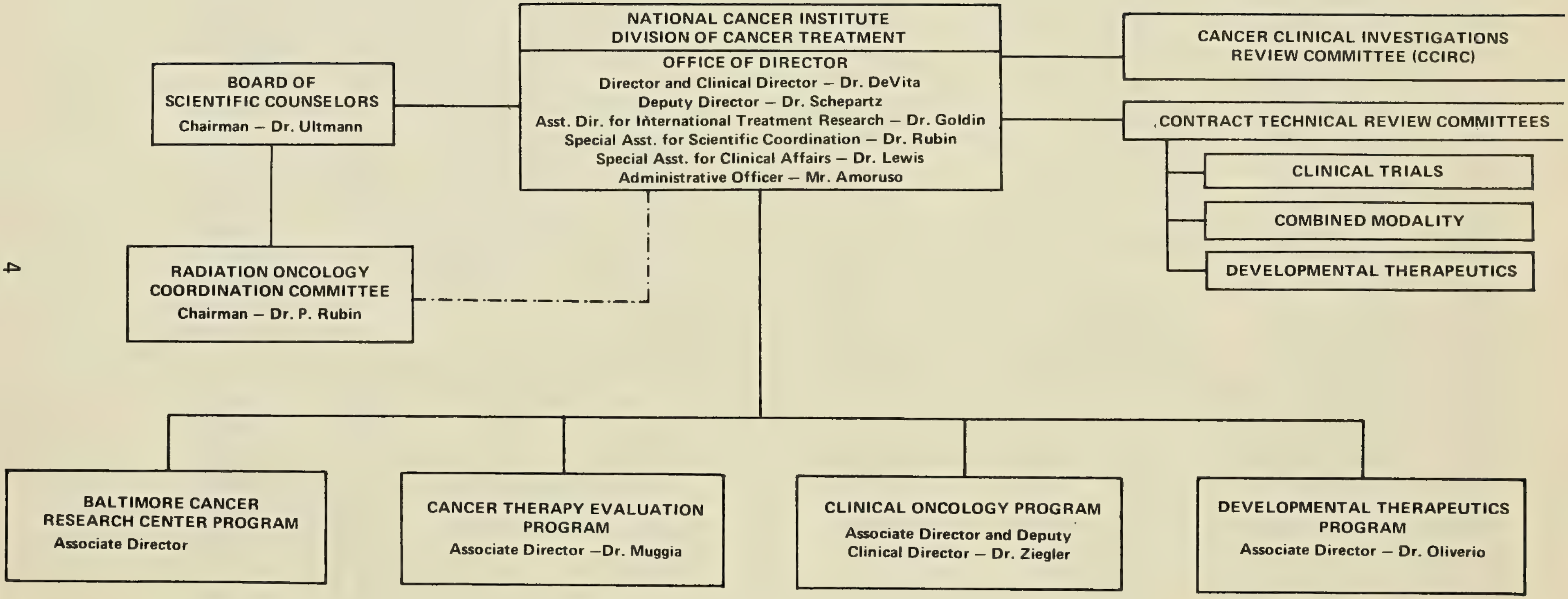
In July, 1975, the Director, DCT, established a radiation oncology subcommittee of the DCT Board of Scientific Counselors, the Radiation Oncology Coordination Subcommittee (ROCS). The organization of the ROCS and its relationship within the Division of Cancer Treatment are given in Figures I and II. As shown, the ROCS functions both as a subcommittee of the DCT Board of Scientific Counselors and as advisor directly to the Office of the Director, DCT, on radiation oncology research.

The Radiation Oncology Coordination Subcommittee has been delegated the principal responsibility to provide expert advice to the DCT in the planning and development of the radiation oncology research program. The scientific basis for the planning activities of the ROCS is the "Research Plan for Radiation Oncology" (prepared by the Committee for Radiation Oncology Studies, vide infra) and the associated NCI publication "Digest of Scientific Recommendations: Radiation Oncology." The responsibility of the ROCS includes:

- -- A review and assessment of the state of the science in radiation oncology;
- -- Development of a scientific plan and recommendations for future research activities to be submitted to the Division of Cancer Treatment and its Board of Scientific Counselors for consideration for implementation by the Program; and,
- -- Assistance in fostering collaborative research among investigators within the program in the area of radiation oncology and therapy.

The organization of the ROCS is shown in Figure II. The Executive Council is composed of three groups. The Executive Nucleus is comprised of the radiotherapists who are members of the Board of Scientific Counselors, one of whom is appointed the Chairman of ROCS; the DCT Scientific Coordinator for Radiation Oncology, representing the Office of the Director, DCT, and an Executive Secretary appointed by the Chairman of ROCS. There are also two ad hoc members of the Executive Council representing the Chairman of the Committee for Radiation Oncology Studies (CROS), supported by the Division of Research Resources and Centers, and the Radiation Therapy Oncology Group (RTOG), a clinical cooperative group supported by the DCT. The second group within the Executive Council consists of the working group coordinators, whose responsibilities are described below. In addition, another group of ad hoc members to the ROCS is composed of all the radiation oncologists on the technical review committees of the DCT.

Specifically, as advisors to the Division of Cancer Treatment on research planning in the area of radiation oncology, the ROCS and its working groups have the responsibilities of: assessing the state of the science; setting objectives for various radiation oncology research areas; identifying gaps and recommending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CONTRACT TECHNICAL REVIEW COMMITTEES
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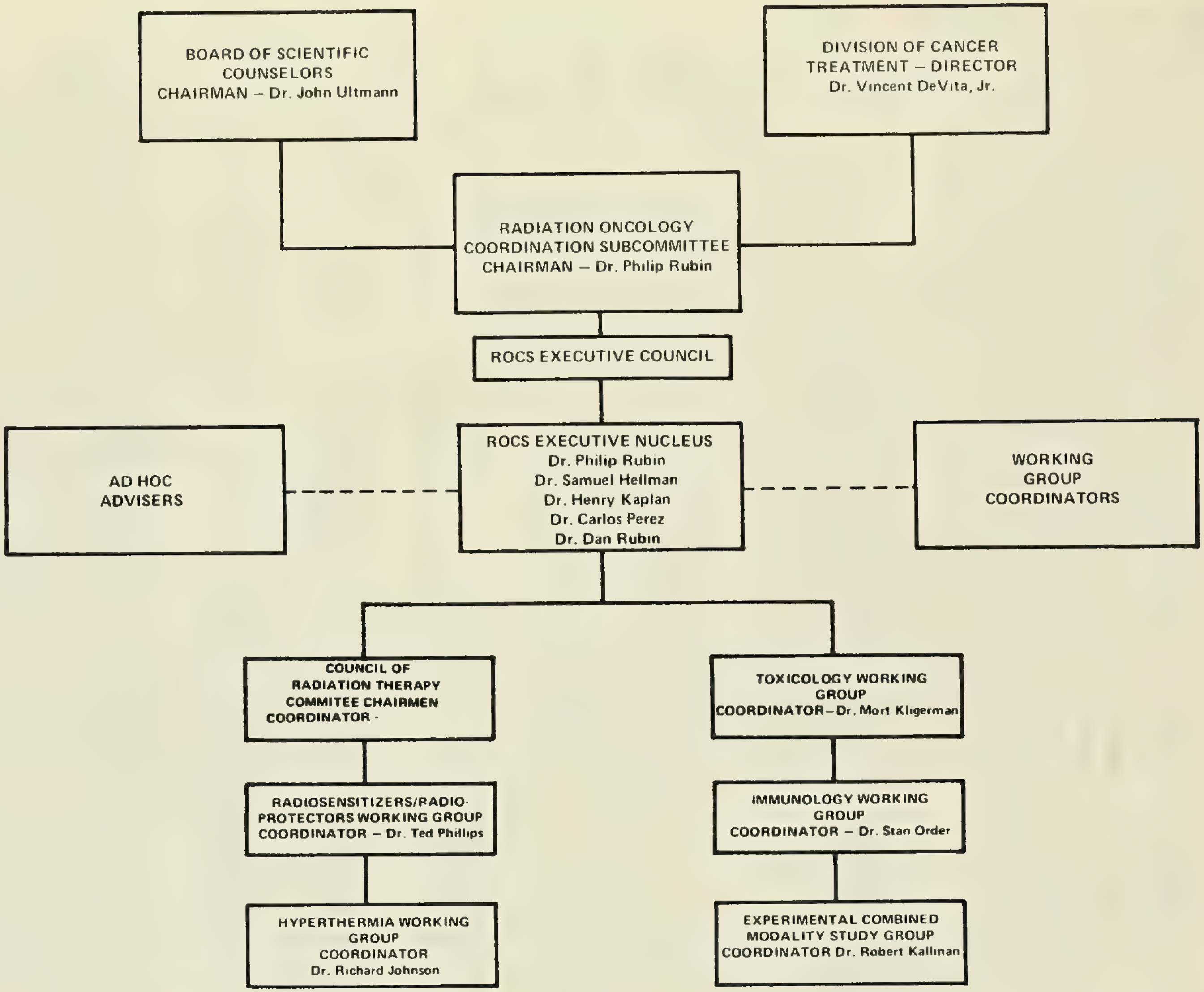
CLINICAL ONCOLOGY PROGRAM
Associate Director and Deputy Clinical Director – Dr. Ziegler
DEVELOPMENTAL THERAPEUTICS PROGRAM
Associate Director – Dr. Oliverio

FIGURE I. DIVISION OF CANCER TREATMENT ORGANIZATION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COORDINATOR – Dr. Ted Phillips
IMMUNOLOGY WORKING GROUP
COORDINATOR – Dr. Stan Order
HYPERTHERMIA WORKING GROUP
COORDINATOR
Dr. Richard Johnson
EXPERIMENTAL COMBINED MODALITY STUDY GROUP
COORDINATOR Dr. Robert Kallman

FIGURE II. RADIATION ONCOLOGY COORDINATION SUBCOMMITTEE ORGANIZATION

needed research; as well as assessing progress in research on radiation oncology and providing estimates of future resource requirements to the program.

The radiotherapists on the Board of Scientific Counselors are automatically appointed to the ROCS. With the exception of the ROCS Chairman and the DCT Coordinator, who are appointed by the Director, DCT, all of the members of the ROCS are appointed by the Chairman. Working groups are determined by the major clinical and preclinical research areas of radiation oncology that are to be planned. Currently, six research areas have been identified and the following working groups have been or are in the process of being established. They are:

- The Radiosensitizers/Radioprotectors Working Group is responsible for developing plans and scientific recommendations for preclinical and/or clinical research in radioprotectors, radiochemical modifiers and hypoxic cell sensitizers that enhance the efficacy of radiotherapy. Secondly, this working group has assumed the responsibility of a specialized subcommittee to the DCT Drug Development Decision Network. As such, it will participate in the selection, evaluation of data on, and the testing of radiosensitizer compounds, and make recommendations regarding research and development on various classes of compounds which enhance the therapeutic efficacy of radiation. In concert with the DCT staff, the Committee has also initiated the development of a research logic for the Decision Network Linear Array to cover research and development of radiosensitizer compounds.

- The Hyperthermia Working Group is responsible for developing plans and scientific recommendations for preclinical and/or clinical research in hyperthermia combined with radiation therapy.

- The Toxicology Working Group is responsible for developing plans and scientific recommendations for preclinical and/or clinical research on the toxicology of radiotherapy.

- The Immunology Working Group is responsible for developing plans and scientific recommendations for preclinical and/or clinical research in combined immunotherapy/radiotherapy.

- The Experimental Combined Modalities Study Group is responsible for developing plans and scientific recommendations for preclinical combined modality (chemotherapy/radiotherapy) research. This group is primarily composed of recent recipients of Cancer Research Emphasis Grants (DCT-3) in this research area. They have also agreed to constitute a collaborative research study group both in assuming responsibilities of the Subcommittee of ROCS, as well as in promoting the exchange of scientific information and the collaborative approaches to their research.

- The Council of Radiotherapy Chairmen of the Clinical Cooperative Groups is responsible for developing plans and scientific recommendations for clinical trials research in radiotherapy. This group is selected from radiotherapy chairmen or chief consultants of the Clinical Cooperative Groups or major task force.

In the past year major progress has been made with the assistance of the Radiation Oncology Coordination Subcommittee, through its reports to the Board of Scientific Counselors and the Director, DCT, in the continued development and expansion of radiation oncology research at both preclinical and clinical levels that reflect our continuing effort to achieve total program balance within the Division. This has been reflected both in the area of scientific information exchange and collaboration, such as the workshop on radiosensitizers held under the auspices of this subcommittee as well as in the expanding research being supported by the various programs within DCT.

In an effort to exploit the increased sensitivity of malignant cells to heat, the DCT has sponsored several projects in hyperthermia research. Phase I testing of a whole-body hyperthermia suit has been successfully completed. In this study, it was possible to safely maintain patients with advanced cancer at 41.8 degrees Centigrade for four hours. The next step will be to attempt to augment the effects of chemotherapy by administering it during periods of hyperthermia.

In parallel with this work, the DCT has developed more accurate temperature monitoring to increase the safety and accuracy of the heating process. We have also encouraged and assisted the Radiation Therapy Oncology Group in designing clinical trials and in identifying equipment for testing the effects of local hyperthermia upon cirumscribed tumors. At the same time, the DCT has critically analyzed and reviewed the use of microwaves for local heat induction and has awarded a CREG to catalyze the development and the clinical application of this method of heating tissue.

During the past year continued emphasis has been placed on the potential utility of combined modality therapy, especially in the clinical portions of the program. The encouraging results obtained in breast cancer in particular, have resulted in increased emphasis and effort within the cooperative groups, within clinical contract areas, and intramurally. It is expected that this trend will continue during the coming year. Specific details of the progress and highlights within each of the program areas are contained in the Summary Report of each Associate Director and will not be repeated here.

The Office of the Assistant Director for International Treatment Research, headed by Dr. Abraham Goldin, was established in 1976. The international activities encompass the interests of the various program areas of the Division and their relationships to international treatment research. These activities are coordinated closely with the Office of the Associate Director for International Affairs, OD, NCI. This involves the development and conduct of collaborative preclinical and clinical programs, exchange of personnel, procurement and exchange of synthetic agents and natural products for screening and development, and the identification of new compounds of potential clinical interest.

All in all, the Division of Cancer Treatment represents the complete array of basic research in areas related to developmental therapeutics, all the steps intervening between the identification of basic leads up to clinical trials and the inclusion of clinical trials to improve the treatment of patients with cancer with the existing technologies, and to develop new technologies for the futures.

Division of Cancer Treatment Stratification

Code	Description
PDD	Preclinical Drug Development Program -
1XX	Stage I - Acquisition of Materials
2A1	Stage II - Basic Screen - Determination of anti-tumor activity of new agents.
2B1	- Develop acceptable experimental formulation.
2B2	- Verification Screen - Detailed evaluation of new agents -- dose, route, and schedule dependency.
2B3	- Procurement of sufficient amounts of new agent for preclinical studies.
3X1	Stage III - Toxicology and pharmacology in large animals.
3X2	- Production and formulation for clinical trials.
PBR	Preclinical Basic Research -
PBS	Biological Studies - Includes biochemistry, blood products, cell biology, cell kinetics, nutrition, immunobiology, markers, molecular biology, radiobiology, transfusion research, data processing
PTS	Treatment Studies - Independent treatments, combined modality therapy
CTR	Clinical Trials Research -
CT1	Phase I Clinical Trials* - initial clinical evaluation of new drugs, clinical pharmacology.
CT2	Phase II Clinical Trials* - allocation for specific disease-oriented resources to study whatever chemotherapy (single agents or combinations) or combined modality regimens have highest priority for initial efficacy evaluation.
CT3	Phase III Clinical Trials* - allocation for specific disease-oriented resources to study whatever chemotherapy or combined modality regimens have highest priority for efficacy evaluation in a controlled clinical setting.

CT4	Phase IV Clinical Trials[*] - allocation for specific disease-oriented resources to evaluate the combined modality approach to the initial therapeutic attack on local or regional disease in an attempt to increase the number of patients with a long disease-free period.
CT5	Statistics, data processing, other clinical trials research.
CSR	Clinical Trials Supportive Research - Includes Special Pharm./Tox., cell kinetics, markers, blood products, transfusion research, protected environment, hyperthemia, nutrition, statistics, data processing.
MGT	Program Management - Includes administration, dissemination of information to medical and scientific community.

[*] Supportive care used as ancillary therapy should be prorated among the phases of clinical trials using such resources.

TABLE I - ANALYSIS OF CONTRACT ACTIVITIES FOR T.Q.

	T.Q. LEVEL *	PERCENT
PDD PRECLINICAL DRUG DEVELOPMENT PROGRAM	7,975,339	71.00
STAGE I	1,737,618	15.47
1XX ACQUISITION OF MATERIALS		
STAGE IIA	2,938,795	26.16
2A1 DETERMINATION OF ANTI-TUMOR ACTIVITY		
STAGE IIB	1,678,725	14.94
2B1 EXPERIMENTAL FORMULATION DEVELOPMENT	66,704	0.59
2B2 DETAILED EVALUATION OF NEW AGENTS	1,029,223	9.16
2B3 PROCURE. OF AGENTS FOR PRECLIN. STUDIES	582,798	5.19
STAGE III	1,620,201	14.42
3X1 TOXICOLOGY & PHARM. IN LARGE ANIMALS	152,157	1.35
3X2 PROD. & FORM. FOR CLINICAL TRIALS	1,468,044	13.07
PBR PRECLINICAL BASIC RESEARCH	372,187	3.31
PBS BIOLOGICAL STUDIES	265,721	2.37
PTS TREATMENT STUDIES	106,466	0.95
CTR CLINICAL TRIALS RESEARCH	1,547,718	13.78
CT1 PHASE I CLINICAL TRIALS	122,882	1.09
CT2 PHASE II CLINICAL TRIALS	377,682	3.36
CT3 PHASE III CLINICAL TRIALS	712,761	6.35
CT4 PHASE IV CLINICAL TRIALS	334,393	2.98
CSR CLINICAL TRIALS SUPPORTIVE RESEARCH	1,209,261	10.77
MGT PROGRAM MANAGEMENT	128,610	1.14
TOTAL	11,233,115	100.00

* TRANSITIONAL QUARTER FUNDING REQUIREMENTS FOR 70 CONTRACTS. NOT INCLUDED ARE FUNDS UTILIZED FOR DIRECT PURCHASE OF CLINICAL DRUGS ($650,000).

TABLE II

ANALYSIS OF CONTRACTS BY ACTIVITY

ACTIVITY

FOR TRANSITIONAL QUARTER
07/01/76 - 09/30/76

CONTRACT	#	DOLLAR LEVEL
STAGE I ACQUISITION OF MATERIALS:		
ABBOTT LABORATORIES	N01CM43704	21,305
AGRICULTURE, DEPARTMENT OF	Y01CM40001	117,000
ARIZONA, UNIVERSITY OF	N01CM33750	60,000
ARMY, DEPARTMENT OF, FT. DETRICK	Y01CO50004	38,058
ARS/SPRAGUE-DAWLEY	N01CM53813	162,519
ARTHUR D. LITTLE, INC.	N01CM57006	750
BRISTOL-MYERS COMPANY	N01CM43759	49,000
CHARLES RIVER BREEDING LABS.	N01CM53812	80,000
CHARLES RIVER BREEDING LABS.	N01CM53848	3,300
COLLABORATIVE RESEARCH, INC.	N01CM60506	40,414
HAZLETON LABORATORIES, INC.	N01CM33708	3,000
KANSAS, UNIVERSITY OF	N01CM53819	19,588
LEO GOODWIN INST. FOR CANCER RESEARCH	N01CM53814	79,593
MICROBIOLOGICAL ASSOCIATES, INC.	N01CM53818	29,340
PFIZER, INC.	N01CM53805	312,589
POMONA COLLEGE	N01CM67062	47,782
RUTGERS STATE UNIVERSITY	N01CM67106	104,871

CONTRACT	#	DOLLAR LEVEL
STAGE I ACQUISITION OF MATERIALS:		
SIMONSEN LABORATORIES	N01CM53817	51,946
SMALL BUSINESS ADMINISTRATION	N01CM43719	46,392
TEXAS INBRED MICE	N01CM53816	83,971
TULANE UNIVERSITY	N01CM53815	36,241
UPJOHN COMPANY	N01CM43753	97,109
VALUE ENGINEERING COMPANY	N01CM67086	128,401
VETERANS ADMINISTRATION HOSPITAL	Y01CM60102	10,396
WARF INSTITUTE, INC.	N01CM43707	113,459
TOTAL		1,737,624

ANALYSIS OF CONTRACTS BY ACTIVITY

ACTIVITY

FOR TRANSITIONAL QUARTER
07/01/76 - 09/30/76

CONTRACT	#	DOLLAR LEVEL
STAGE II BASIC SCREEN:		
ARMY, DEPARTMENT OF, FT. DETRICK	Y01CO50004	9,515
ARS/SPRAGUE-DAWLEY	N01CM53813	447,202
ARTHUR D. LITTLE, INC.	N01CM57006	14,250
ARTHUR D. LITTLE, INC.	N01CM53765	17,445
BATTELLE MEMORIAL INSTITUTE	N01CM67099	428,842
CATHOLIC MEDICAL CTR OF BROOKLYN AND QUEENS	N01CM67081	2,189
CHARLES RIVER BREEDING LABS.	N01CM53812	220,000
CHARLES RIVER BREEDING LABS.	N01CM53848	10,450
KANSAS, UNIVERSITY OF	N01CM53819	53,869
LEO GOODWIN INST. FOR CANCER RESEARCH	N01CM53814	218,881
MICHIGAN DEPARTMENT OF PUBLIC HEALTH	N01CM33702	69,693
MICROBIOLOGICAL ASSOCIATES, INC.	N01CM53818	80,685
SIMONSEN LABORATORIES	N01CM53817	142,850
STEHLIN FOUNDATION	N01CM67073	317,810
TEXAS INBRED MICE	N01CM53816	230,921
TULANE UNIVERSITY	N01CM53815	99,663
UPJOHN COMPANY	N01CM43753	28,963

CONTRACT	#	DOLLAR LEVEL
STAGE II BASIC SCREEN:		
VALUE ENGINEERING COMPANY	N01CM67086	531,946
YALE UNIVERSITY SCHOOL OF MEDICINE	N01CM67068	13,619
TOTAL		2,938,793

ANALYSIS OF CONTRACTS BY ACTIVITY

ACTIVITY

FOR TRANSITIONAL QUARTER
07/01/76 - 09/30/76

CONTRACT	#	DOLLAR LEVEL
STAGE II BIOLOGICAL RESEARCH PROGRAMS:		
ARIZONA, UNIVERSITY OF	N01CM33713	12,314
HAZLETON LABORATORIES, INC.	N01CM33708	4,500
HAZLETON LABORATORIES, INC.	N01CM43702	229,024
SMALL BUSINESS ADMINISTRATION	N01CM43719	19,883
TOTAL		265,721

ANALYSIS OF CONTRACTS BY ACTIVITY

ACTIVITY

FOR TRANSITIONAL QUARTER
07/01/76 - 09/30/76

CONTRACT	#	DOLLAR LEVEL
STAGE II FORMULATION:		
BEN VENUE LABORATORIES, INC.	N01CM67109	65,000
UPJOHN COMPANY	N01CM43753	1,704
TOTAL		66,704

ANALYSIS OF CONTRACTS BY ACTIVITY

ACTIVITY

FOR TRANSITIONAL QUARTER
07/01/76 - 09/30/76

CONTRACT	#	DOLLAR LEVEL
STAGE II VERIFICATION SCREEN:		
ARMY, DEPARTMENT OF, FT. DETRICK	Y01CO50004	23,787
ARS/SPRAGUE-DAWLEY	N01CM53813	203,274
ARTHUR D. LITTLE, INC.	N01CM57006	7,500
ARTHUR D. LITTLE, INC.	N01CM53765	9,968
CATHOLIC MEDICAL CTR OF BROOKLYN AND QUEENS	N01CM67081	12,045
CHARLES RIVER BREEDING LABS.	N01CM53812	100,000
CHARLES RIVER BREEDING LABS.	N01CM53848	4,750
HAZLETON LABORATORIES, INC.	N01CM33708	1,500
KANSAS, UNIVERSITY OF	N01CM53819	24,486
LEO GOODWIN INST. FOR CANCER RESEARCH	N01CM53814	99,492
MADRID, UNIVERSITY OF	N01CM53792	31,762
MICHIGAN CANCER FOUNDATION	N01CM53789	58,321
MICROBIOLOGICAL ASSOCIATES, INC.	N01CM53818	36,675
SIMONSEN LABORATORIES	N01CM53817	64,932
TEXAS INBRED MICE	N01CM53816	104,954
TULANE UNIVERSITY	N01CM53815	45,301
UPJOHN COMPANY	N01CM43753	17,037

CONTRACT	#	DOLLAR LEVEL
STAGE II VERIFICATION SCREEN:		
VALUE ENGINEERING COMPANY	N01CM67086	183,430
TOTAL		1,029,224

ANALYSIS OF CONTRACTS BY ACTIVITY

ACTIVITY

FOR TRANSITIONAL QUARTER
07/01/76 - 09/30/76

CONTRACT	#	DOLLAR LEVEL
STAGE II PROCUREMENT OF PRECLIN. MAT'L:		
AGRICULTURE, DEPARTMENT OF	Y01CM40001	13,000
ARMY, DEPARTMENT OF, FT. DETRICK	Y01CO50004	7,929
COLLABORATIVE RESEARCH, INC.	N01CM60506	4,490
MONSANTO RESEARCH CORP.	N01CM67046	67,201
MONSANTO RESEARCH CORPORATION	N01CM23715	90,045
PARKE, DAVIS AND COMPANY	N01CM23708	25,652
RESEARCH TRIANGLE INSTITUTE	N01CM67121	165,604
STANFORD RESEARCH INSTITUTE	N01CM12162	19,964
STANFORD RESEARCH INSTITUTE	N01CM63776	187,757
VETERANS ADMINISTRATION HOSPITAL	Y01CM60102	1,155
TOTAL		582,797

ANALYSIS OF CONTRACTS BY ACTIVITY

ACTIVITY

FOR TRANSITIONAL QUARTER
07/01/76 - 09/30/76

CONTRACT	#	DOLLAR LEVEL
STAGE III PHARMACOLOGY/TOXICOLOGY:		
HAZLETON LABORATORIES, INC.	N01CM33708	6,000
PUBLIC HEALTH SERVICE	Y01CM40003	13,980
SOUTHERN CALIFORNIA, UNIVERSITY OF	N01CM67065	90,733
UPJOHN COMPANY	N01CM43753	25,555
YALE UNIVERSITY SCHOOL OF MEDICINE	N01CM67068	15,888
TOTAL		152,156

ANALYSIS OF CONTRACTS BY ACTIVITY

ACTIVITY

FOR TRANSITIONAL QUARTER
07/01/76 - 09/30/76

CONTRACT	#	DOLLAR LEVEL
STAGE III PRECLIN. COMBINED TREATMENT:		
ARMY RES. INST. OF ENVIRONMENTAL MEDICINE	Y01CM60105	500
ARTHUR D. LITTLE, INC.	N01CM57006	2,500
ARTHUR D. LITTLE, INC.	N01CM53765	22,429
CATHOLIC MEDICAL CTR OF BROOKLYN AND QUEENS	N01CM67081	7,665
VALUE ENGINEERING COMPANY	N01CM67086	73,372
TOTAL		106,466

ANALYSIS OF CONTRACTS BY ACTIVITY

ACTIVITY

FOR TRANSITIONAL QUARTER
07/01/76 - 09/30/76

CONTRACT	#	DOLLAR LEVEL
STAGE III PROD./FORM. FOR CLIN. TRIALS:		
BEN VENUE LABORATORIES, INC.	N01CM67109	585,000
FLOW LABORATORIES	N01CM67088	136,734
MONSANTO RESEARCH CORP.	N01CM67046	604,816
MONSANTO RESEARCH CORPORATION	N01CM23715	22,511
PARKE, DAVIS AND COMPANY	N01CM23708	25,652
RESEARCH TRIANGLE INSTITUTE	N01CM67121	41,401
STANFORD RESEARCH INSTITUTE	N01CM12162	4,991
STANFORD RESEARCH INSTITUTE	N01CM63776	46,939
TOTAL		1,468,044

ANALYSIS OF CONTRACTS BY ACTIVITY

ACTIVITY

FOR TRANSITIONAL QUARTER
07/01/76 - 09/30/76

CONTRACT	#	DOLLAR LEVEL
PROGRAM MANAGEMENT:		
ABBOTT LABORATORIES	N01CM43704	14,202
PUBLIC HEALTH SERVICE	Y01CM40003	2,330
VALUE ENGINEERING COMPANY	N01CM53821	112,078
TOTAL		128,610

ANALYSIS OF CONTRACTS BY ACTIVITY

ACTIVITY

FOR TRANSITIONAL QUARTER
07/01/76 - 09/30/76

CONTRACT	#	DOLLAR LEVEL
PHASE I CLINICAL TRIALS:		
NATIONAL NAVAL MEDICAL CENTER	Y01CM50100	1,106
PUBLIC HEALTH SERVICE	Y01CM40003	16,310
VETERANS ADMINISTRATION	Y01CM40004	88,500
WALTER REED ARMY MEDICAL CENTER	Y01CM40005	1,085
YALE UNIVERSITY SCHOOL OF MEDICINE	N01CM67068	15,889
TOTAL		122,890

ANALYSIS OF CONTRACTS BY ACTIVITY

ACTIVITY

FOR TRANSITIONAL QUARTER
07/01/76 - 09/30/76

CONTRACT	#	DOLLAR LEVEL
PHASE II CLINICAL TRIALS:		
ARIZONA, UNIVERSITY OF	N01CM33713	36,943
MAYO FOUNDATION	N01CM02066	126,278
MIAMI, UNIVERSITY OF	N01CM43800	300
NATIONAL NAVAL MEDICAL CENTER	Y01CM50100	2,370
TEXAS, UNIVERSITY OF, SYSTEM CANCER CTR/MDA	N01CM57042	67,522
VETERANS ADMINISTRATION	Y01CM40004	177,000
WALTER REED ARMY MEDICAL CENTER	Y01CM40005	5,140
TOTAL		415,553

ANALYSIS OF CONTRACTS BY ACTIVITY

ACTIVITY

FOR TRANSITIONAL QUARTER
07/01/76 - 09/30/76

CONTRACT	#	DOLLAR LEVEL
PHASE III CLINICAL TRIALS:		
CALIFORNIA, UNIVERSITY OF	N01HB62971	55,266
COMMUNITY BLOOD & PLASMA SERVICE	N01CL42150	479,810
HEALTH RESEARCH, INC.	N01CM67117	60,652
IOWA, UNIVERSTIY OF	N01HB62973	50,830
MAYO FOUNDATION	N01CM02066	42,092
MAYO FOUNDATION	N01CM67123	92,522
MICROBIOLOGICAL ASSOCIATES, INC.	N01CM63810	100,000
MINNESOTA, UNIVERSITY OF	N01HB62972	51,160
NATIONAL NAVAL MEDICAL CENTER	Y01CM50100	3,002
TEXAS, UNIVERSITY OF, SYSTEM CANCER CTR/MDA	N01CM57042	67,530
VETERANS ADMINISTRATION	Y01CM40004	442,500
WALTER REED ARMY MEDICAL CENTER	Y01CM40005	4,463
WASHINGTON UNIVERSITY	N01HB62970	44,177
TOTAL		1,494,004

ANALYSIS OF CONTRACTS BY ACTIVITY

ACTIVITY

FOR TRANSITIONAL QUARTER
07/01/76 - 09/30/76

CONTRACT	#	DOLLAR LEVEL
PHASE IV CLINICAL TRIALS (COMB. MODAL.):		
HEALTH RESEARCH, INC.	N01CM67117	60,651
MAYO FOUNDATION	N01CM67123	92,523
MIAMI, UNIVERSITY OF	N01CM43800	1,200
NATIONAL NAVAL MEDICAL CENTER	Y01CM50100	1,264
VETERANS ADMINISTRATION	Y01CM40004	177,000
WALTER REED ARMY MEDICAL CENTER	Y01CM40005	1,755
TOTAL		334,393

ANALYSIS OF CONTRACTS BY ACTIVITY

FOR TRANSITIONAL QUARTER
07/01/76 - 09/30/76

ACTIVITY CONTRACT	#	DOLLAR LEVEL
D.O.S. PHARMACOLOGY/TOXICOLOGY:		
NATIONAL NAVAL MEDICAL CENTER	Y01CM50100	158
PUBLIC HEALTH SERVICE	Y01CM40003	13,980
SOUTHERN CALIFORNIA, UNIVERSITY OF	N01CM67065	24,196
TOTAL		38,334

ANALYSIS OF CONTRACTS BY ACTIVITY

ACTIVITY

FOR TRANSITIONAL QUARTER
07/01/76 - 09/30/76

CONTRACT	#	DOLLAR LEVEL
D.O.S. DATA PROCESSING:		
ARIZONA, UNIVERSITY OF	N01CM33713	12,314
HEALTH RESEARCH, INC.	N01CM67114	16,775
INSTITUTO NAZIONALE LA CURA DEI TOMORI	N01CM67115	6,681
MINNESOTA, UNIVERSITY OF	N01CM67113	12,243
NEW ENGLAND MEDICAL CENTER	N01CM67112	5,886
SOUTHERN CALIFORNIA, UNIVERSITY OF	N01CM67065	6,049
STANFORD UNIVERSITY, BOARD OF TRUSTEES	N01CM67111	19,838
STANFORD UNIVERSITY, BOARD OF TRUSTEES	N01CM67072	21,668
WALTER REED ARMY MEDICAL CENTER	Y01CM40005	1,082
TOTAL		102,536

ANALYSIS OF CONTRACTS BY ACTIVITY

ACTIVITY

FOR TRANSITIONAL QUARTER
07/01/76 - 09/30/76

CONTRACT	#	DOLLAR LEVEL
D.O.S. OTHER:		
HEALTH RESEARCH, INC.	N01CM67114	50,325
INSTITUTO NAZIONALE LA CURA DEI TOMORI	N01CM67115	20,042
MINNESOTA, UNIVERSITY OF	N01CM67113	36,729
NEW ENGLAND MEDICAL CENTER	N01CM67112	17,657
STANFORD UNIVERSITY, BOARD OF TRUSTEES	N01CM67111	59,513
STANFORD UNIVERSITY, BOARD OF TRUSTEES	N01CM67072	65,003
TOTAL		249,269

TABLE III

ANALYSIS OF DIVISION OF CANCER TREATMENT

ACTIVITIES BY CONTRACT

FOR TRANSITIONAL QUARTER
07/01/76 - 09/30/76

CONTRACTOR/FUNCTIONS	#	PERCENT OF EFFORT		DOLLAR LEVEL
ABBOTT LABORATORIES	N01CM43704			35,507
1XX ACQUISITION OF MATERIALS:		* 60*	21,305	
05 BIOASSAY OF NATURAL PRODUCTS		24	8,522	
98 DATA PROCESSING AND SUPPORT		16	5,681	
99 OTHER-HUMAN CELL LINES		20	7,101	
XXX PROGRAM MANAGEMENT:		* 40*	14,202	
01 ADMINISTRATION		20	7,101	
98 DATA PROCESSING AND SUPPORT		20	7,101	
AGRICULTURE, DEPARTMENT OF	Y01CM40001			130,000
1XX ACQUISITION OF MATERIALS:		* 90*	117,000	
02 NEW AGENT PROCUREMENT, PLANT PRODUCTS		85	110,500	
98 DATA PROCESSING AND SUPPORT		5	6,500	
2B3 PROCUREMENT OF PRECLIN. MAT'L:		* 10*	13,000	
02 PLANT PRODUCTS				
ARIZONA, UNIVERSITY OF	N01CM33713			61,571
2A2 BIOLOGICAL RESEARCH PROGRAMS:		* 20*	12,314	
04 MARKERS				
PHASE II CLINICAL TRIALS:		* 60*	36,943	
16 MYELOMA	12 MARKERS			
D.O.S. DATA PROCESSING:		* 20*	12,314	
16 MYELOMA	25 DATA PROCESSING			
ARIZONA, UNIVERSITY OF	N01CM33750			60,000
1XX ACQUISITION OF MATERIALS:		*100*	60,000	
02 NEW AGENT PROCUREMENT, PLANT PRODUCTS		79	47,400	
03 NEW AGENT PROCURE., FERMENT./ANTIBIOTICS		10	6,000	
05 BIOASSAY OF NATURAL PRODUCTS		11	6,600	
ARMY RES. INST. OF ENVIRONMENTAL	Y01CM60105			500
3X2 PRECLIN. COMBINED TREATMENT:		*100*	500	
99 OTHER-HYPERTHERMIA				
ARMY, DEPARTMENT OF, FT. DETRICK	Y01C050004			79,289
1XX ACQUISITION OF MATERIALS:		* 48*	38,058	
03 NEW AGENT PROCURE., FERMENT./ANTIBIOTICS				
2A1 BASIC SCREEN:		* 12*	9,515	
01 PRIMARY SCREENING, IN VITRO		3	2,379	
04 ANIMALS		9	7,136	

CONTRACTOR/FUNCTIONS	#	PERCENT OF EFFORT		DOLLAR LEVEL
2B2 VERIFICATION SCREEN:		* 30*	23,787	
04 ANIMALS				
2B3 PROCUREMENT OF PRECLIN. MAT'L:		* 10*	7,929	
03 FERMENTATION/ANTIBIOTICS				
ARS/SPRAGUE-DAWLEY	N01CM53813			813,095
1XX ACQUISITION OF MATERIALS:		* 20*	162,619	
06 ANIMALS				
2A1 BASIC SCREEN:		* 55*	447,202	
04 ANIMALS				
2B2 VERIFICATION SCREEN:		* 25*	203,274	
04 ANIMALS				
ARTHUR D. LITTLE, INC.	N01CM57006			25,000
1XX ACQUISITION OF MATERIALS:		* 3*	750	
05 BIOASSAY OF NATURAL PRODUCTS				
2A1 BASIC SCREEN:		* 57*	14,250	
02 PRIMARY SCREENING, IN VIVO		50	12,500	
03 PRIMARY SCREENING, REL. NEW MODEL DEVEL.		7	1,750	
2B2 VERIFICATION SCREEN:		* 30*	7,500	
02 DETAILED DRUG EVALUATION, IN VIVO		25	6,250	
03 DETAILED DRUG EVAL., REL. NEW MODEL DEV.		5	1,250	
3X2 PRECLIN. COMBINED TREATMENT:		* 10*	2,500	
01 COMBINATION CHEMOTHERAPY				
ARTHUR D. LITTLE, INC.	N01CM53765			49,842
2A1 BASIC SCREEN:		* 35*	17,445	
02 PRIMARY SCREENING, IN VIVO				
2B2 VERIFICATION SCREEN:		* 20*	9,968	
02 DETAILED DRUG EVALUATION, IN VIVO				
3X2 PRECLIN. COMBINED TREATMENT:		* 45*	22,429	
01 COMBINATION CHEMOTHERAPY		27	13,457	
02 COMBINED MODALITY PROGRAM		18	8,972	
BATTELLE MEMORIAL INSTITUTE	N01CM67099			428,842
2A1 BASIC SCREEN:		*100*	428,842	
02 PRIMARY SCREENING, IN VIVO		75	321,632	
03 PRIMARY SCREENING, REL. NEW MODEL DEVEL.		25	107,211	

CONTRACTOR/FUNCTIONS	#	PERCENT OF EFFORT		DOLLAR LEVEL
BEN VENUE LABORATORIES, INC.	N01CM67109			650,000
2B1 FORMULATION:		* 10*	65,000	
01 DEVEL. OF EXPERIMENTAL FORMULATIONS				
3X3 PROD./FORM. FOR CLIN. TRIALS:		* 90*	585,000	
06 FORMULATION		75	487,500	
07 ANALYTICAL AND QUALITY CONTROL		15	97,500	
BRISTOL-MYERS COMPANY	N01CM43759			49,000
1XX ACQUISITION OF MATERIALS:		*100*	49,000	
03 NEW AGENT PROCURE., FERMENT./ANTIBIOTICS				
CALIFORNIA, UNIVERSITY OF	N01HB62971			55,266
PHASE III CLINICAL TRIALS:		*100*	55,266	
12 LEUKEMIA 18 TRANSF. RESEARCH				
CATHOLIC MEDICAL CTR OF BROOKLYN AND	N01CM67081			21,899
2A1 BASIC SCREEN:		* 10*	2,189	
02 PRIMARY SCREENING, IN VIVO				
2B2 VERIFICATION SCREEN:		* 55*	12,045	
02 DETAILED DRUG EVALUATION, IN VIVO		50	10,950	
03 DETAILED DRUG EVAL., REL. NEW MODEL DEV.		5	1,095	
3X2 PRECLIN. COMBINED TREATMENT:		* 35*	7,665	
01 COMBINATION CHEMOTHERAPY		25	5,475	
02 COMBINED MODALITY PROGRAM		10	2,190	
CHARLES RIVER BREEDING LABS.	N01CM53812			400,000
1XX ACQUISITION OF MATERIALS:		* 20*	80,000	
06 ANIMALS				
2A1 BASIC SCREEN:		* 55*	220,000	
04 ANIMALS				
2B2 VERIFICATION SCREEN:		* 25*	100,000	
04 ANIMALS				
CHARLES RIVER BREEDING LABS.	N01CM53848			19,000
1XX ACQUISITION OF MATERIALS:		* 20*	3,800	
06 ANIMALS				
2A1 BASIC SCREEN:		* 55*	10,450	
04 ANIMALS				
2B2 VERIFICATION SCREEN:		* 25*	4,750	
04 ANIMALS				

CONTRACTOR/FUNCTIONS	#	PERCENT OF EFFORT		DOLLAR LEVEL
COLLABORATIVE RESEARCH, INC.	N01CM60506			44,904
1XX ACQUISITION OF MATERIALS:		* 90*	40,414	
01 NEW AGENT PROCUREMENT, SYNTHETICS				
2B3 PROCUREMENT OF PRECLIN. MAT'L:		* 10*	4,490	
01 SYNTHETICS				
COMMUNITY BLOOD & PLASMA SERVICE	N01CL42150			479,810
PHASE III CLINICAL TRIALS:		*100*	479,810	
12 LEUKEMIA	17 BLOOD PROD.	80	383,848	
14 LYMPHOMAS	17 BLOOD PROD.	10	47,981	
99 OTHER-APLASIA	17 BLOOD PROD.	10	47,981	
FLOW LABORATORIES	N01CM67088			136,734
3X3 PROD./FORM. FOR CLIN. TRIALS:		*100*	136,734	
99 OTHER-STOR.&DIST. OF DRUGS				
HAZLETON LABORATORIES, INC.	N01CM33708			15,000
1XX ACQUISITION OF MATERIALS:		* 20*	3,000	
06 ANIMALS				
2A2 BIOLOGICAL RESEARCH PROGRAMS:		* 30*	4,500	
04 MARKERS				
2B2 VERIFICATION SCREEN:		* 10*	1,500	
04 ANIMALS				
3X1 PHARMACOLOGY/TOXICOLOGY:		* 40*	6,000	
01 PHARMACOLOGY		10	1,500	
03 SPECIAL TOXICITY STUDIES		30	4,500	
HAZLETON LABORATORIES, INC.	N01CM43702			229,024
2A2 BIOLOGICAL RESEARCH PROGRAMS:		*100*	229,024	
06 TRANS. RESEARCH		50	114,512	
99 OTHER-TRANSPLANTATION		50	114,512	
HEALTH RESEARCH, INC.	N01CM67114			67,100
D.O.S. DATA PROCESSING:		* 25*	16,775	
14 LYMPHOMAS	25 DATA PROCESSING			
D.O.S. OTHER:		* 75*	50,325	
14 LYMPHOMAS	99 OTHER-PATH.DIAGNOS			
HEALTH RESEARCH, INC.	N01CM67117			121,303
PHASE III CLINICAL TRIALS:		* 50*	60,652	
17 OVARY	13 CHEM.	20	24,261	
17 OVARY	14 SURG.	15	18,195	

CONTRACTOR/FUNCTIONS		#	PERCENT OF EFFORT		DOLLAR LEVEL
17 OVARY	15 RAD.		15	18,195	
PHASE IV CLINICAL TRIALS (COMB. MODAL.):			* 50*	60,651	
17 OVARY	20 CHEM.		20	24,261	
17 OVARY	21 SURG.		15	18,195	
17 OVARY	22 RAD.		15	18,195	
INSTITUTO NAZIONALE LA CURA DEI TOMORI		N01CM67115			26,723
D.O.S. DATA PROCESSING:			* 25*	6,681	
14 LYMPHOMAS	25 DATA PROCESSING				
D.O.S. OTHER:			* 75*	20,042	
14 LYMPHOMAS	99 OTHER-PATH.DIAGNOS				
IOWA, UNIVERSTIY OF		N01HB62973			50,830
PHASE III CLINICAL TRIALS:			*100*	50,830	
12 LEUKEMIA	18 TRANSF. RESEARCH				
KANSAS, UNIVERSITY OF		N01CM53819			97,943
1XX ACQUISITION OF MATERIALS:			* 20*	19,588	
06 ANIMALS					
2A1 BASIC SCREEN:			* 55*	53,869	
04 ANIMALS					
2B2 VERIFICATION SCREEN:			* 25*	24,486	
04 ANIMALS					
LEO GOODWIN INST. FOR CANCER RESEARCH		N01CM53814			397,966
1XX ACQUISITION OF MATERIALS:			* 20*	79,593	
06 ANIMALS					
2A1 BASIC SCREEN:			* 55*	218,881	
04 ANIMALS					
2B2 VERIFICATION SCREEN:			* 25*	99,492	
04 ANIMALS					
MADRID, UNIVERSITY OF		N01CM53792			31,762
2B2 VERIFICATION SCREEN:			*100*	31,762	
01 DETAILED DRUG EVALUATION, IN VITRO			70	22,233	
02 DETAILED DRUG EVALUATION, IN VIVO			10	3,176	
03 DETAILED DRUG EVAL., REL. NEW MODEL DEV.			20	6,352	
MAYO FOUNDATION		N01CM02066			168,370
PHASE II CLINICAL TRIALS:			* 75*	126,278	
06 COLON	07 CHEM.		60	101,022	

CONTRACTOR/FUNCTIONS		#	PERCENT OF EFFORT		DOLLAR LEVEL
18 PANCREAS	07 CHEM.		7	11,786	
22 STOMACH	07 CHEM.		8	13,470	
PHASE III CLINICAL TRIALS:			* 25*	42,092	
06 COLON	13 CHEM.		20	33,674	
18 PANCREAS	13 CHEM.		3	5,051	
22 STOMACH	13 CHEM.		2	3,367	
MAYO FOUNDATION		N01CM67123			185,045
PHASE III CLINICAL TRIALS:			* 50*	92,522	
17 OVARY	13 CHEM.		20	37,009	
17 OVARY	14 SURG.		15	27,757	
17 OVARY	15 RAD.		15	27,757	
PHASE IV CLINICAL TRIALS (COMB. MODAL.):			* 50*	92,523	
17 OVARY	20 CHEM.		20	37,009	
17 OVARY	21 SURG.		15	27,757	
17 OVARY	22 RAD.		15	27,757	
MIAMI, UNIVERSITY OF		N01CM43800			1,500
PHASE II CLINICAL TRIALS:			* 20*	300	
18 PANCREAS	07 CHEM.				
PHASE IV CLINICAL TRIALS (COMB. MODAL.):			* 80*	1,200	
18 PANCREAS	20 CHEM.		26	390	
18 PANCREAS	21 SURG.		28	420	
18 PANCREAS	22 RAD.		26	390	
MICHIGAN CANCER FOUNDATION		N01CM53789			58,321
2B2 VERIFICATION SCREEN:			*100*	58,321	
01 DETAILED DRUG EVALUATION, IN VITRO			80	46,657	
03 DETAILED DRUG EVAL., REL. NEW MODEL DEV.			20	11,664	
MICHIGAN DEPARTMENT OF PUBLIC HEALTH		N01CM33702			69,693
2A1 BASIC SCREEN:			*100*	69,693	
01 PRIMARY SCREENING, IN VITRO					
MICROBIOLOGICAL ASSOCIATES, INC.		N01CM63810			100,000
PHASE III CLINICAL TRIALS:			*100*	100,000	
12 LEUKEMIA	18 TRANSF. RESEARCH				
MICROBIOLOGICAL ASSOCIATES, INC.		N01CM53818			146,700
1XX ACQUISITION OF MATERIALS:			* 20*	29,340	
06 ANIMALS					

CONTRACTOR/FUNCTIONS	#	PERCENT OF EFFORT		DOLLAR LEVEL
2A1 BASIC SCREEN:		* 55*	80,685	
04 ANIMALS				
2B2 VERIFICATION SCREEN:		* 25*	36,675	
04 ANIMALS				
MINNESOTA, UNIVERSITY OF	N01CM67113			48,972
D.O.S. DATA PROCESSING:		* 25*	12,243	
14 LYMPHOMAS	25 DATA PROCESSING			
D.O.S. OTHER:		* 75*	36,729	
14 LYMPHOMAS	99 OTHER-PATH.DIAGNOS			
MINNESOTA, UNIVERSITY OF	N01HB62972			51,160
PHASE III CLINICAL TRIALS:		*100*	51,160	
12 LEUKEMIA	18 TRANSF. RESEARCH			
MONSANTO RESEARCH CORP.	N01CM67046			672,017
2B3 PROCUREMENT OF PRECLIN. MAT'L:		* 10*	67,201	
02 PLANT PRODUCTS				
3X3 PROD./FORM. FOR CLIN. TRIALS:		* 90*	604,816	
01 PRODUCTION, SYNTHETICS		15	100,803	
02 PRODUCTION, PLANT PRODUCTS		75	504,013	
MONSANTO RESEARCH CORPORATION	N01CM23715			112,556
2B3 PROCUREMENT OF PRECLIN. MAT'L:		* 80*	90,045	
05 RADIOLABELED MATERIALS				
3X3 PROD./FORM. FOR CLIN. TRIALS:		* 20*	22,511	
99 OTHER-RADIOLABEL				
NATIONAL NAVAL MEDICAL CENTER	Y01CM50100			7,900
PHASE I CLINICAL TRIALS:		* 14*	1,106	
04 BREAST	01 CHEM.			
PHASE II CLINICAL TRIALS:		* 30*	2,370	
04 BREAST	07 CHEM.	25	1,975	
04 BREAST	12 MARKERS	5	395	
PHASE III CLINICAL TRIALS:		* 38*	3,002	
04 BREAST	13 CHEM.			
PHASE IV CLINICAL TRIALS (COMB. MODAL.):		* 16*	1,264	
04 BREAST	20 CHEM.	6	474	
04 BREAST	21 SURG.	6	474	
04 BREAST	23 IMM.	4	316	

CONTRACTOR/FUNCTIONS	#	PERCENT OF EFFORT		DOLLAR LEVEL
D.O.S. PHARMACOLOGY/TOXICOLOGY:		* 2*	158	
04 BREAST	24 PHARM./TOX.			
NEW ENGLAND MEDICAL CENTER	N01CM67112			23,542
D.O.S. DATA PROCESSING:		* 25*	5,886	
14 LYMPHOMAS	25 DATA PROCESSING			
D.O.S. OTHER:		* 75*	17,657	
14 LYMPHOMAS	99 OTHER-PATH.DIAGNOS			
PARKE, DAVIS AND COMPANY	N01CM23708			51,304
2B3 PROCUREMENT OF PRECLIN. MAT'L:		* 50*	25,652	
01 SYNTHETICS				
3X3 PROD./FORM. FOR CLIN. TRIALS:		* 50*	25,652	
01 PRODUCTION, SYNTHETICS		25	12,826	
02 PRODUCTION, PLANT PRODUCTS		25	12,826	
PFIZER, INC.	N01CM53805			312,589
1XX ACQUISITION OF MATERIALS:		*100*	312,589	
99 OTHER-ANIMAL VIRUSES				
POMONA COLLEGE	N01CM67062			47,782
1XX ACQUISITION OF MATERIALS:		*100*	47,782	
99 OTHER-STRUCTURE ACTIVITY				
PUBLIC HEALTH SERVICE	Y01CM40003			46,600
3X1 PHARMACOLOGY/TOXICOLOGY:		* 30*	13,980	
01 PHARMACOLOGY				
XXX PROGRAM MANAGEMENT:		* 5*	2,330	
01 ADMINISTRATION				
PHASE I CLINICAL TRIALS:		* 35*	16,310	
01 NONSPECIFIC	04 IMM.	15	6,990	
01 NONSPECIFIC	05 PHARM./TOX.	20	9,320	
D.O.S. PHARMACOLOGY/TOXICOLOGY:		* 30*	13,980	
01 NONSPECIFIC	24 PHARM./TOX.			
RESEARCH TRIANGLE INSTITUTE	N01CM67121			207,005
2B3 PROCUREMENT OF PRECLIN. MAT'L:		* 80*	165,604	
05 RADIOLABELED MATERIALS				
3X3 PROD./FORM. FOR CLIN. TRIALS:		* 20*	41,401	
99 OTHER-RADIOLABEL				

CONTRACTOR/FUNCTIONS	#	PERCENT OF EFFORT		DOLLAR LEVEL
RUTGERS STATE UNIVERSITY	N01CM67106			104,871
1XX ACQUISITION OF MATERIALS:		*100*	104,871	
03 NEW AGENT PROCURE., FERMENT./ANTIBIOTICS				
SIMONSEN LABORATORIES	N01CM53817			259,728
1XX ACQUISITION OF MATERIALS:		* 20*	51,946	
06 ANIMALS				
2A1 BASIC SCREEN:		* 55*	142,850	
04 ANIMALS				
2B2 VERIFICATION SCREEN:		* 25*	64,932	
04 ANIMALS				
SMALL BUSINESS ADMINISTRATION	N01CM43719			66,275
1XX ACQUISITION OF MATERIALS:		* 70*	46,392	
99 OTHER-ANIMAL CELLS				
2A2 BIOLOGICAL RESEARCH PROGRAMS:		* 30*	19,883	
01 MOLECULAR BIOLOGY		10	6,628	
99 OTHER-RADIOIMMUNE ASSAY		20	13,255	
SOUTHERN CALIFORNIA, UNIVERSITY OF	N01CM67065			120,978
3X1 PHARMACOLOGY/TOXICOLOGY:		* 75*	90,733	
01 PHARMACOLOGY		65	78,636	
98 DATA PROCESSING AND SUPPORT		10	12,098	
D.O.S. PHARMACOLOGY/TOXICOLOGY:		* 20*	24,196	
01 NONSPECIFIC	24 PHARM./TOX.			
D.O.S. DATA PROCESSING:		* 5*	6,049	
01 NONSPECIFIC	25 DATA PROCESSING			
STANFORD RESEARCH INSTITUTE	N01CM12162			24,955
2B3 PROCUREMENT OF PRECLIN. MAT'L:		* 80*	19,964	
05 RADIOLABELED MATERIALS				
3X3 PROD./FORM. FOR CLIN. TRIALS:		* 20*	4,991	
99 OTHER-RADIOLABEL				
STANFORD RESEARCH INSTITUTE	N01CM63776			234,696
2B3 PROCUREMENT OF PRECLIN. MAT'L:		* 80*	187,757	
05 RADIOLABELED MATERIALS				
3X3 PROD./FORM. FOR CLIN. TRIALS:		* 20*	46,939	
99 OTHER-RADIOLABEL				
STANFORD UNIVERSITY, BOARD OF TRUSTEES	N01CM67111			79,350
D.O.S. DATA PROCESSING:		* 25*	19,838	

CONTRACTOR/FUNCTIONS	#	PERCENT OF EFFORT		DOLLAR LEVEL
14 LYMPHOMAS	25 DATA PROCESSING			
D.O.S. OTHER:		* 75*	59,513	
14 LYMPHOMAS	99 OTHER-PATH.DIAGNOS			
STANFORD UNIVERSITY, BOARD OF TRUSTEES	N01CM67072			86,670
D.O.S. DATA PROCESSING:		* 25*	21,668	
14 LYMPHOMAS	25 DATA PROCESSING			
D.O.S. OTHER:		* 75*	65,003	
14 LYMPHOMAS	99 OTHER-PATH.DIAGNOS			
STEHLIN FOUNDATION	N01CM67073			317,810
2A1 BASIC SCREEN:		*100*	317,810	
03 PRIMARY SCREENING, REL. NEW MODEL DEVEL.				
TEXAS INBRED MICE	N01CM53816			419,856
1XX ACQUISITION OF MATERIALS:		* 20*	83,971	
06 ANIMALS				
2A1 BASIC SCREEN:		* 55*	230,921	
04 ANIMALS				
2B2 VERIFICATION SCREEN:		* 25*	104,964	
04 ANIMALS				
TEXAS, UNIVERSITY OF, SYSTEM CANCER	N01CM57042			135,052
PHASE II CLINICAL TRIALS:		* 50*	67,522	
02 BLADDER	07 CHEM.	2	2,701	
04 BREAST	07 CHEM.	5	6,753	
05 CERVIX	07 CHEM.	2	2,701	
06 COLON	07 CHEM.	5	6,753	
09 HEAD & NECK	07 CHEM.	2	2,701	
11 KIDNEY	07 CHEM.	3	4,052	
13 LUNG	07 CHEM.	5	6,753	
15 MELANOMA	07 CHEM.	3	4,052	
17 OVARY	07 CHEM.	5	6,753	
18 PANCREAS	07 CHEM.	5	6,753	
20 PROSTATE	07 CHEM.	5	6,753	
21 SARCOMAS	07 CHEM.	5	6,753	
22 STOMACH	07 CHEM.	3	4,052	
PHASE III CLINICAL TRIALS:		* 50*	67,530	
02 BLADDER	13 CHEM.	3	4,052	

CONTRACTOR/FUNCTIONS	#	PERCENT OF EFFORT		DOLLAR LEVEL
04 BREAST	13 CHEM.	5	6,753	
05 CERVIX	13 CHEM.	3	4,052	
06 COLON	13 CHEM.	5	6,753	
09 HEAD & NECK	13 CHEM.	3	4,052	
11 KIDNEY	13 CHEM.	2	2,701	
13 LUNG	13 CHEM.	5	6,753	
15 MELANOMA	13 CHEM.	2	2,701	
17 OVARY	13 CHEM.	5	6,753	
18 PANCREAS	13 CHEM.	5	6,753	
20 PROSTATE	13 CHEM.	5	6,753	
21 SARCOMAS	13 CHEM.	5	6,753	
22 STOMACH	13 CHEM.	2	2,701	
TULANE UNIVERSITY	N01CM53815			181,205
1XX ACQUISITION OF MATERIALS:		* 20*	36,241	
06 ANIMALS				
2A1 BASIC SCREEN:		* 55*	99,663	
04 ANIMALS				
2B2 VERIFICATION SCREEN:		* 25*	45,301	
04 ANIMALS				
UPJOHN COMPANY	N01CM43753			170,368
1XX ACQUISITION OF MATERIALS:		* 57*	97,109	
03 NEW AGENT PROCURE., FERMENT./ANTIBIOTICS		55	93,702	
98 DATA PROCESSING AND SUPPORT		2	3,407	
2A1 BASIC SCREEN:		* 17*	28,963	
01 PRIMARY SCREENING, IN VITRO		7	11,926	
02 PRIMARY SCREENING, IN VIVO		3	5,111	
03 PRIMARY SCREENING, REL. NEW MODEL DEVEL.		7	11,926	
2B1 FORMULATION:		* 1*	1,704	
01 DEVEL. OF EXPERIMENTAL FORMULATIONS				
2B2 VERIFICATION SCREEN:		* 10*	17,037	
99 OTHER-MOLECULAR BIOLOGY				
3X1 PHARMACOLOGY/TOXICOLOGY:		* 15*	25,555	
01 PHARMACOLOGY		13	22,148	
98 DATA PROCESSING AND SUPPORT		2	3,407	

CONTRACTOR/FUNCTIONS		#	PERCENT OF EFFORT		DOLLAR LEVEL
VALUF ENGINEERING COMPANY		N01CM53821			112,078
XXX PROGRAM MANAGEMENT:			*100*	112,078	
98 DATA PROCESSING AND SUPPORT					
VALUE ENGINEERING COMPANY		N01CM67086			917,149
1XX ACQUISITION OF MATERIALS:			* 14*	128,401	
05 BIOASSAY OF NATURAL PRODUCTS					
2A1 BASIC SCREEN:			* 58*	531,946	
01 PRIMARY SCREENING, IN VITRO			5	45,857	
02 PRIMARY SCREENING, IN VIVO			53	486,089	
2B2 VERIFICATION SCREEN:			* 20*	183,430	
02 DETAILED DRUG EVALUATION, IN VIVO					
3X2 PRECLIN. COMBINED TREATMENT:			* 8*	73,372	
01 COMBINATION CHEMOTHERAPY					
VETERANS ADMINISTRATION		Y01CM40004			885,000
PHASE I CLINICAL TRIALS:			* 10*	88,500	
01 NONSPECIFIC	01 CHEM.				
PHASE II CLINICAL TRIALS:			* 20*	177,000	
06 COLON	07 CHEM.		1	8,850	
13 LUNG	07 CHEM.		15	132,750	
18 PANCREAS	07 CHEM.		1	8,850	
20 PROSTATE	07 CHEM.		2	17,700	
22 STOMACH	07 CHEM.		1	8,850	
PHASE III CLINICAL TRIALS:			* 50*	442,500	
06 COLON	13 CHEM.		3	26,550	
13 LUNG	13 CHEM.		20	177,000	
13 LUNG	15 RAD.		15	132,750	
18 PANCREAS	13 CHEM.		4	35,400	
20 PROSTATE	13 CHEM.		5	44,250	
22 STOMACH	13 CHEM.		3	26,550	
PHASE IV CLINICAL TRIALS (COMB. MODAL.):			* 20*	177,000	
06 COLON	20 CHEM.		1	8,850	
06 COLON	21 SURG.		1	8,850	
13 LUNG	20 CHEM.		7	61,950	
13 LUNG	21 SURG.		8	70,800	
18 PANCREAS	20 CHEM.		1	8,850	
18 PANCREAS	21 SURG.		1	8,850	

CONTRACTOR/FUNCTIONS	#	PERCENT OF EFFORT		DOLLAR LEVEL
22 STOMACH	20 CHEM.	1	8,850	
VETERANS ADMINISTRATION	Y01CM60102			11,551
1XX ACQUISITION OF MATERIALS:		* 90*	10,396	
01 NEW AGENT PROCUREMENT, SYNTHETICS				
2B3 PROCUREMENT OF PRECLIN. MAT'L:		* 10*	1,155	
01 SYNTHETICS				
WALTER REED ARMY MEDICAL CENTER	Y01CM40005			13,525
PHASE I CLINICAL TRIALS:		* 8*	1,085	
04 BREAST	01 CHEM.			
PHASE II CLINICAL TRIALS:		* 38*	5,140	
04 BREAST	07 CHEM.	34	4,599	
04 BREAST	12 MARKERS	4	541	
PHASE III CLINICAL TRIALS:		* 33*	4,463	
04 BREAST	13 CHEM.	13	1,758	
12 LEUKEMIA	13 CHEM.	5	676	
14 LYMPHOMAS	13 CHEM.	8	1,082	
14 LYMPHOMAS	15 RAD.	2	271	
23 TESTICULAR	13 CHEM.	5	676	
PHASE IV CLINICAL TRIALS (COMB. MODAL.):		* 13*	1,755	
04 BREAST	20 CHEM.	1	135	
04 BREAST	21 SURG.	1	135	
04 BREAST	22 RAD.	1	135	
04 BREAST	23 IMM.	1	135	
13 LUNG	20 CHEM.	1	135	
13 LUNG	21 SURG.	1	135	
13 LUNG	22 RAD.	1	135	
13 LUNG	23 IMM.	1	135	
14 LYMPHOMAS	20 CHEM.	1	135	
14 LYMPHOMAS	22 RAD.	1	135	
23 TESTICULAR	20 CHEM.	1	135	
23 TESTICULAR	21 SURG.	1	135	
23 TESTICULAR	22 RAD.	1	135	
D.O.S. DATA PROCESSING:		* 8*	1,082	
01 NONSPECIFIC	25 DATA PROCESSING			

CONTRACTOR/FUNCTIONS	#	PERCENT OF EFFORT		DOLLAR LEVEL
WARF INSTITUTE, INC.	N01CM43707			113,459
1XX ACQUISITION OF MATERIALS:		*100*	113,459	
02 NEW AGENT PROCUREMENT, PLANT PRODUCTS				
WASHINGTON UNIVERSITY	N01HB62970			44,177
PHASE III CLINICAL TRIALS:		*100*	44,177	
12 LEUKEMIA	18 TRANSF. RESEARCH			
YALE UNIVERSITY SCHOOL OF MEDICINE	N01CM67068			45,396
2A1 BASIC SCREEN:		* 30*	13,619	
01 PRIMARY SCREENING, IN VITRO				
3X1 PHARMACOLOGY/TOXICOLOGY:		* 35*	15,888	
01 PHARMACOLOGY		20	9,079	
99 OTHER-		15	6,809	
PHASE I CLINICAL TRIALS:		* 35*	15,889	
01 NONSPECIFIC	05 PHARM./TOX.			

TABLE IV - ANALYSIS OF CONTRACT ACTIVITIES FOR FY77

	ANNUAL LEVEL *	PERCENT
PDD PRECLINICAL DRUG DEVELOPMENT PROGRAM	41,665,270	58.61
STAGE I	13,748,463	19.34
1XX ACQUISITION OF MATERIALS		
STAGE IIA	11,702,464	16.46
2A1 DETERMINATION OF ANTI-TUMOR ACTIVITY		
STAGE IIB	10,449,983	14.70
2B1 EXPERIMENTAL FORMULATION DEVELOPMENT	306,560	0.43
2B2 DETAILED EVALUATION OF NEW AGENTS	6,813,022	9.58
2B3 PROCURE. OF AGENTS FOR PRECLIN. STUDIES	3,330,401	4.69
STAGE III	5,764,360	8.11
3X1 TOXICOLOGY & PHARM. IN LARGE ANIMALS	2,482,757	3.49
3X2 PROD. & FORM. FOR CLINICAL TRIALS	3,281,603	4.62
PBR PRECLINICAL BASIC RESEARCH	5,473,510	7.70
PBS BIOLOGICAL STUDIES	3,090,805	4.35
PTS TREATMENT STUDIES	2,382,705	3.35
CTR CLINICAL TRIALS RESEARCH	17,614,673	24.78
CT1 PHASE I CLINICAL TRIALS	1,561,033	2.20
CT2 PHASE II CLINICAL TRIALS	3,864,264	5.44
CT3 PHASE III CLINICAL TRIALS	5,792,035	8.15
CT4 PHASE IV CLINICAL TRIALS	5,470,286	7.70
CT5 OTHER CLINICAL TRIALS RESEARCH	927,055	1.30
CSR CLINICAL TRIALS SUPPORTIVE RESEARCH	3,973,354	5.59
MGT PROGRAM MANAGEMENT	2,356,556	3.32
TOTAL	71,083,363	100.00

* FY-77 FUNDING REQUIREMENTS AS OF MAY 31, 1977 FOR APPROXIMATELY 293 CONTRACTS. INCLUDED ARE APPROXIMATELY 10 SCHEDULE A CONTRACTS ($1,937,560) AND 8 SCHEDULE B CONTRACTS ($945,000). NOT INCLUDED ARE FUNDS UTILIZED FOR DIRECT PURCHASE OF CLINICAL DRUGS (APPROXIMATELY $3,000,000).

TABLE V

ANALYSIS OF CONTRACTS BY ACTIVITY

ACTIVITY FOR FISCAL YEAR 1977
AS OF 05/31/77

AREA	CONTRACT	#	DOLLAR LEVEL
	STAGE I AQUISITION OF MATERIALS:		
D.T.P.	AGRICULTURE, DEPARTMENT OF	Y01CM40001	370,800
A.P.	ALABAMA, UNIVERSITY OF	N01CM67083	21,104
D.T.P.	ARIZONA STATE UNIVERSITY	N01CM67048	125,019
O.D.	ARMY, DEPARTMENT OF, FT. DETRICK	Y01CO60603	174,227
A.P.	ARS/SPRAGUE-DAWLEY	N01CM53813	200,000
A.P.	ARS/SPRAGUE-DAWLEY	N01CM53846	35,000
D.T.P.	ARTHUR D. LITTLE, INC.	N01CM57006	17,454
O.D.	ARTHUR D. LITTLE, INC.	N01CM63823	32,967
D.T.P.	ARTHUR D. LITTLE, INC.	N01CM67078	10,770
D.T.P.	ASSOCIATED BIOMEDIC SYSTEMS, INC.	N01CM02271	150,000
D.T.P.	BATTELLE MEMORIAL INSTITUTE	N01CM57005	116,397
A.P.	BELLAIRE ACRES	N01CM50583	43,368
D.T.P.	BRISTOL-MYERS COMPANY	N01CM77147	283,050
D.T.P.	BRITISH COLUMBIA, UNIVERSITY OF	N01CM23223	87,988
A.P.	CHARLES RIVER BREEDING LABS.	N01CM50598	50,232
A.P.	CHARLES RIVER BREEDING LABS.	N01CM53812	310,000
A.P.	CHARLES RIVER BREEDING LABS.	N01CM53848	21,600
D.T.P.	CHEMICAL ABSTRACTS SERVICE	N01CM43722	449,100
D.T.P.	CHESTER BEATTY RESEARCH INSTITUTE	N01CM43736	16,000
D.T.P.	COLLABORATIVE RESEARCH, INC.	N01CM77071	84,844
D.T.P.	FARMITALIA	N01CM57014	173,847
D.T.P.	FARMITALIA	N01CM57036	170,631
A.P.	FLOW LABORATORIES, INC.	N01CM50571	43,992
D.T.P.	GEORGETOWN UNIVERSITY	N01CM43732	59,985

ANALYSIS OF CONTRACTS BY ACTIVITY

ACTIVITY

FOR FISCAL YEAR 1977
AS OF 05/31/77

AREA	CONTRACT	#	DOLLAR LEVEL
	STAGE I AQUISITION OF MATERIALS:		
D.T.P.	GRACE, W.R.	N01CM67074	221,182
A.P.	HARLAN INDUSTRIES	N01CM50591	45,240
D.T.P.	HAWAII, UNIVERSITY OF	N01CM33747	82,246
D.T.P.	HAZLETON LABORATORIES, INC.	N01CM33708	80,001
A.P.	HEALTH RESEARCH, INC.	N01CM77101	17,194
D.T.P.	IIT RESEARCH INSTITUTE	N01CM43755	56,974
A.P.	IIT RESEARCH INSTITUTE	N01CM53829	25,813
D.T.P.	IIT RESEARCH INSTITUTE	N01CM57004	129,758
D.T.P.	IIT RESEARCH INSTITUTE	N01CM63832	4,108
D.T.P.	ILLINOIS, UNIVERSITY OF	N01CM67090	96,134
D.T.P.	INSTITUT JULES BORDET	N01CM53840	28,170
A.P.	JACKSON LABORATORY	N01CM60176	30,732
D.T.P.	JAPANESE FOUNDATION FOR CANCER RESEARCH	N01CM22054	7,050
A.P.	LABORATORY SUPPLY CO.	N01CM50577	46,176
A.P.	LABORATORY SUPPLY CO.	N01CM53775	28,559
A.P.	LEO GOODWIN INST. FOR CANCER RESEARCH	N01CM53814	96,000
A.P.	LITTON BIONETICS, INC.	N01CM53778	15,261
O.D.	LITTON BIONETICS, INC.	N01CO25423	2,400,000
D.T.P.	MARIO NEGRI INST OF PHARMACOLOGICAL RESEARCH	N01CM33720	28,967
D.T.P.	MASON RESEARCH INSTITUTE	N01CM57003	34,841
D.T.P.	MASSACHUSETTS INSTITUTE OF TECHNOLOGY	N01CM43712	52,800
D.T.P.	MIAMI, UNIVERSITY OF	N01CM67077	6,875
D.T.P.	MICROBIAL CHEMISTRY RESEARCH FDN.	N01CM57009	45,000
D.T.P.	MICROBIOLOGICAL ASSOCIATES, INC.	N01CM43793	278,400

ANALYSIS OF CONTRACTS BY ACTIVITY

ACTIVITY FOR FISCAL YEAR 1977
AS OF 05/31/77

AREA	CONTRACT	#	DOLLAR LEVEL
	STAGE I AQUISITION OF MATERIALS:		
A.P.	MICROBIOLOGICAL ASSOCIATES, INC.	N01CM53818	50,000
A.P.	MICROBIOLOGICAL ASSOCIATES, INC.	N01CM67069	37,520
D.T.P.	MIDWEST RESEARCH INSTITUTE	N01CM33743	316,292
A.P.	MURPHY BREEDING LABS., INC.	N01CM50579	42,432
D.T.P.	NORTHEASTERN UNIVERSITY	N01CM53741	68,743
D.T.P.	OHIO STATE UNIVERSITY RESEARCH FOUNDATION	N01CM57015	61,401
D.T.P.	OKLAHOMA, UNIVERSITY OF	N01CM67108	59,203
D.T.P.	PARKE, DAVIS AND COMPANY	N01CM43754	110,287
D.T.P.	PARKE, DAVIS AND COMPANY	N01CM77146	564,946
D.T.P.	PFIZER, INC.	N01CM53805	289,000
D.T.P.	POLYSCIENCES, INC.	N01CM77070	280,845
D.T.P.	POMONA COLLEGE	N01CM67062	53,000
D.T.P.	PURDUE UNIVERSITY	N01CM67091	106,907
D.T.P.	RESEARCH TRIANGLE INSTITUTE	N01CM67089	161,648
D.T.P.	RUTGERS STATE UNIVERSITY	N01CM67106	98,192
D.T.P.	SAINT JUDE'S CHILDREN'S RESEARCH HOSPITAL	N01CM77127	89,145
A.P.	SIMONSEN LABORATORIES	N01CM50578	44,928
A.P.	SIMONSEN LABORATORIES	N01CM53817	120,000
A.P.	SIMONSEN LABORATORIES	N01CM53847	33,000
D.T.P.	SMALL BUSINESS ADMINISTRATION	N01CM43719	105,340
A.P.	SOUTHERN ANIMAL FARMS	N01CM50599	43,992
A.P.	SOUTHERN ANIMAL FARMS	N01CM53845	25,000
D.T.P.	SOUTHERN CALIFORNIA, UNIVERSITY OF	N01CM67084	131,038
D.T.P.	SOUTHERN RESEARCH INSTITUTE	N01CM43756	22,880

ANALYSIS OF CONTRACTS BY ACTIVITY

ACTIVITY FOR FISCAL YEAR 1977
AS OF 05/31/77

AREA	CONTRACT	#	DOLLAR LEVEL
	STAGE I AQUISITION OF MATERIALS:		
D.T.P.	SOUTHERN RESEARCH INSTITUTE	N01CM43762	553,593
D.T.P.	SOUTHWEST FOUNDATION FOR RESEARCH & EDUCATION	N01CM67075	5,303
A.P.	SPARTAN RESEARCH ANIMALS, INC.	N01CM50582	21,840
D.T.P.	STANFORD RESEARCH INSTITUTE	N01CM33742	381,685
D.T.P.	STARKS ASSOCIATES, INC.	N01CM53745	410,404
A.P.	TACONIC FARMS	N01CM50597	35,360
D.T.P.	UPJOHN COMPANY	N01CM43753	250,928
D.T.P.	UPJOHN COMPANY	N01CM77100	669,079
D.T.P.	VALUE ENGINEERING COMPANY	N01CM67086	131,074
D.T.P.	VETERANS ADMINISTRATION HOSPITAL	Y01CM60102	45,094
D.T.P.	VIRGINIA, UNIVERSITY OF	N01CM67002	134,269
D.T.P.	WARF INSTITUTE, INC.	N01CM43707	120,666
D.T.P.	WARF INSTITUTE, INC.	N01CM53770	348,451
D.T.P.	WISCONSIN, UNIVERSITY OF	N01CM67076	2,981
D.T.P.	YALE UNIVERSITY SCHOOL OF MEDICINE	N01CM53824	46,804
D.T.P.	YALE UNIVERSITY SCHOOL OF MEDICINE	N01CM57029	74,333
		TOTAL	13,053,474

ANALYSIS OF CONTRACTS BY ACTIVITY

ACTIVITY

FOR FISCAL YEAR 1977
AS OF 05/31/77

AREA	CONTRACT	#	DOLLAR LEVEL
	STAGE II BASIC SCREEN:		
A.P.	ALABAMA, UNIVERSITY OF	N01CM67083	58,036
O.D.	ARMY, DEPARTMENT OF, FT. DETRICK	Y01CO60603	43,557
A.P.	ARS/SPRAGUE-DAWLEY	N01CM53813	550,000
A.P.	ARS/SPRAGUE-DAWLEY	N01CM53846	96,250
D.T.P.	ARTHUR D. LITTLE, INC.	N01CM53765	448,817
D.T.P.	ARTHUR D. LITTLE, INC.	N01CM53849	63,958
D.T.P.	ARTHUR D. LITTLE, INC.	N01CM57006	418,905
D.T.P.	ARTHUR D. LITTLE, INC.	N01CM67078	204,629
D.T.P.	BATTELLE MEMORIAL INSTITUTE	N01CM57005	426,790
D.T.P.	BATTELLE MEMORIAL INSTITUTE	N01CM67099	454,410
A.P.	BELLAIRE ACRES	N01CM50583	119,262
D.T.P.	BIOTECH RESEARCH LABORATORIES, INC.	N01CM67052	69,000
D.T.P.	BRISTOL-MYERS COMPANY	N01CM77147	49,950
D.T.P.	CATHOLIC MEDICAL CTR OF BROOKLYN AND QUEENS	N01CM67081	205,840
A.P.	CHARLES RIVER BREEDING LABS.	N01CM50598	138,138
A.P.	CHARLES RIVER BREEDING LABS.	N01CM53812	852,500
A.P.	CHARLES RIVER BREEDING LABS.	N01CM53848	59,400
D.T.P.	COLORADO, UNIVERSITY OF, MEDICAL CENTER	N01CM12186	37,500
A.P.	FLOW LABORATORIES, INC.	N01CM50571	120,978
A.P.	HARLAN INDUSTRIES	N01CM50591	124,410
A.P.	HARLAN INDUSTRIES	N01CM67079	136,310
D.T.P.	HEALTH RESEARCH, INC.	N01CM33726	36,188
A.P.	HEALTH RESEARCH, INC.	N01CM77101	47,235
D.T.P.	IIT RESEARCH INSTITUTE	N01CM43755	185,166

ANALYSIS OF CONTRACTS BY ACTIVITY

ACTIVITY FOR FISCAL YEAR 1977
AS OF 05/31/77

AREA	CONTRACT	#	DOLLAR LEVEL
	STAGE II BASIC SCREEN:		
A.P.	IIT RESEARCH INSTITUTE	N01CM53829	70,985
D.T.P.	IIT RESEARCH INSTITUTE	N01CM57004	514,138
D.T.P.	IIT RESEARCH INSTITUTE	N01CM63832	78,043
D.T.P.	INSTITUT JULES BORDET	N01CM57040	230,499
A.P.	JACKSON LABORATORY	N01CM60176	84,513
D.T.P.	JAPANESE FOUNDATION FOR CANCER RESEARCH	N01CM22054	28,200
A.P.	LABORATORY SUPPLY CO.	N01CM50577	126,984
A.P.	LABORATORY SUPPLY CO.	N01CM53775	78,538
A.P.	LEO GOODWIN INST. FOR CANCER RESEARCH	N01CM53814	264,000
A.P.	LITTON BIONETICS, INC.	N01CM53778	41,968
O.D.	LITTON BIONETICS, INC.	N01CO25423	600,000
D.T.P.	MARIO NEGRI INST OF PHARMACOLOGICAL RESEARCH	N01CM33720	135,177
D.T.P.	MASON RESEARCH INSTITUTE	N01CM57003	425,063
D.T.P.	MIAMI, UNIVERSITY OF	N01CM67077	130,633
D.T.P.	MICHIGAN DEPARTMENT OF PUBLIC HEALTH	N01CM33702	124,426
D.T.P.	MICROBIAL CHEMISTRY RESEARCH FDN.	N01CM57009	30,000
A.P.	MICROBIOLOGICAL ASSOCIATES, INC.	N01CM53818	137,500
A.P.	MICROBIOLOGICAL ASSOCIATES, INC.	N01CM67069	103,130
D.T.P.	MIDWEST RESEARCH INSTITUTE	N01CM33743	23,722
A.P.	MURPHY BREEDING LABS., INC.	N01CM50579	116,688
D.T.P.	PARKE, DAVIS AND COMPANY	N01CM43754	19,462
D.T.P.	PARKE, DAVIS AND COMPANY	N01CM77146	99,696
A.P.	SIMONSEN LABORATORIES	N01CM50578	123,552
A.P.	SIMONSEN LABORATORIES	N01CM53817	330,000

ANALYSIS OF CONTRACTS BY ACTIVITY

ACTIVITY	FOR FISCAL YEAR 1977 AS OF 05/31/77		
AREA	CONTRACT	#	DOLLAR LEVEL
	STAGE II BASIC SCREEN:		
A.P.	SIMONSEN LABORATORIES	N01CM53847	90,750
A.P.	SOUTHERN ANIMAL FARMS	N01CM50599	120,978
A.P.	SOUTHERN ANIMAL FARMS	N01CM53845	68,750
D.T.P.	SOUTHERN RESEARCH INSTITUTE	N01CM43756	732,160
D.T.P.	SOUTHWEST FOUNDATION FOR RESEARCH & EDUCATION	N01CM67075	100,845
A.P.	SPARTAN RESEARCH ANIMALS, INC.	N01CM50582	60,060
D.T.P.	STANFORD RESEARCH INSTITUTE	N01CM33742	20,357
D.T.P.	STEHLIN FOUNDATION FOR CANCER RESEARCH	N01CM67073	312,855
A.P.	TACONIC FARMS	N01CM50597	97,240
D.T.P.	UPJOHN COMPANY	N01CM43753	74,838
D.T.P.	UPJOHN COMPANY	N01CM77100	118,073
D.T.P.	VALUE ENGINEERING COMPANY	N01CM67086	543,020
D.T.P.	WARF INSTITUTE, INC.	N01CM53770	278,769
D.T.P.	WISCONSIN, UNIVERSITY OF	N01CM67076	56,643
D.T.P.	YALE UNIVERSITY SCHOOL OF MEDICINE	N01CM53824	23,052
D.T.P.	YALE UNIVERSITY SCHOOL OF MEDICINE	N01CM57029	24,778
D.T.P.	YALE UNIVERSITY SCHOOL OF MEDICINE	N01CM67068	15,000
	TOTAL		11,702,464

ANALYSIS OF CONTRACTS BY ACTIVITY

ACTIVITY FOR FISCAL YEAR 1977
AS OF 05/31/77

AREA	CONTRACT	#	DOLLAR LEVEL
	STAGE II FORMULATION:		
D.T.P.	BEN VENUE LABORATORIES, INC.	N01CM67109	107,787
D.T.P.	ELKINS-SINN, INC.	N01CM67103	29,447
D.T.P.	IOWA, UNIVERSITY OF	N01CM43743	38,700
D.T.P.	KANSAS, UNIVERSITY OF	N01CM23217	20,840
D.T.P.	PHILIPS ROXANE LABORATORIES, INC.	N01CM67053	25,384
D.T.P.	UPJOHN COMPANY	N01CM43753	4,402
	TOTAL		226,560

ANALYSIS OF CONTRACTS BY ACTIVITY

FOR FISCAL YEAR 1977
AS OF 05/31/77

ACTIVITY AREA	CONTRACT	#	DOLLAR LEVEL
	STAGE II VERIFICATION SCREEN:		
A.P.	ALABAMA, UNIVERSITY OF	N01CM67083	26,330
D.T.P.	ALBERT EINSTEIN COLLEGE OF MEDICINE	N01CM43787	74,172
O.D.	ARMY, DEPARTMENT OF, FT. DETRICK	Y01C060603	108,892
A.P.	ARS/SPRAGUE-DAWLEY	N01CM53813	250,000
A.P.	ARS/SPRAGUE-DAWLEY	N01CM53846	43,750
D.T.P.	ARTHUR D. LITTLE, INC.	N01CM57006	145,453
BCRC	ARTHUR D. LITTLE, INC.	N01CM67047	129,913
D.T.P.	BATTELLE MEMORIAL INSTITUTE	N01CM57005	193,995
D.T.P.	BAYLOR COLLEGE OF MEDICINE	N01CM57018	3,040
A.P.	BELLAIRE ACRES	N01CM50583	54,210
D.T.P.	BIOTECH RESEARCH LABORATORIES, INC.	N01CM67052	161,000
D.T.P.	BRIGHAM YOUNG UNIVERSITY	N01CM43790	42,536
D.T.P.	CALIFORNIA, UNIVERSITY OF	N01CM43791	34,804
D.T.P.	CATHOLIC MEDICAL CTR OF BROOKLYN AND QUEENS	N01CM67081	205,840
A.P.	CHARLES RIVER BREEDING LABS.	N01CM50598	62,790
A.P.	CHARLES RIVER BREEDING LABS.	N01CM53812	387,500
A.P.	CHARLES RIVER BREEDING LABS.	N01CM53848	27,000
D.T.P.	CHESTER BEATTY RESEARCH INSTITUTE	N01CM43736	16,000
D.T.P.	ENERGY RES & DEV ADM	Y01CM10056	27,500
A.P.	FLOW LABORATORIES, INC.	N01CM50571	54,990
A.P.	HARLAN INDUSTRIES	N01CM50591	56,550
D.T.P.	HARVARD COLLEGE	N01CM53825	15,556
D.T.P.	HAZLETON LABORATORIES, INC.	N01CM33708	40,001
D.T.P.	HEALTH RESEARCH, INC.	N01CM43785	58,000

ANALYSIS OF CONTRACTS BY ACTIVITY

ACTIVITY FOR FISCAL YEAR 1977
AS OF 05/31/77

AREA	CONTRACT	#	DOLLAR LEVEL
	STAGE II VERIFICATION SCREEN:		
A.P.	HEALTH RESEARCH, INC.	N01CM77101	21,493
D.T.P.	IIT RESEARCH INSTITUTE	N01CM43755	42,731
A.P.	IIT RESEARCH INSTITUTE	N01CM53829	32,266
D.T.P.	IIT RESEARCH INSTITUTE	N01CM57004	95,156
D.T.P.	INSTITUT JULES BORDET	N01CM57040	57,625
A.P.	JACKSON LABORATORY	N01CM60176	38,415
A.P.	LABORATORY SUPPLY CO.	N01CM50577	57,720
A.P.	LABORATORY SUPPLY CO.	N01CM53775	35,699
A.P.	LEO GOODWIN INST. FOR CANCER RESEARCH	N01CM53814	120,000
A.P.	LITTON BIONETICS, INC.	N01CM53778	19,076
O.D.	LITTON BIONETICS, INC.	N01CO25423	1,500,000
D.T.P.	MADRID, UNIVERSITY OF	N01CM53792	18,528
D.T.P.	MARIO NEGRI INST OF PHARMACOLOGICAL RESEARCH	N01CM33720	28,967
D.T.P.	MASON RESEARCH INSTITUTE	N01CM57003	167,238
D.T.P.	MASON RESEARCH INSTITUTE	N01CM57030	166,471
D.T.P.	MICHIGAN CANCER FOUNDATION	N01CM53789	36,450
A.P.	MICROBIOLOGICAL ASSOCIATES, INC.	N01CM53818	62,500
A.P.	MICROBIOLOGICAL ASSOCIATES, INC.	N01CM67069	46,300
D.T.P.	MINNESOTA, UNIVERSITY OF	N01CM43788	53,100
A.P.	MURPHY BREEDING LABS., INC.	N01CM50579	53,040
D.T.P.	PENNSYLVANIA, UNIVERSITY OF	N01CM53830	34,530
A.P.	SIMONSEN LABORATORIES	N01CM50578	56,160
A.P.	SIMONSEN LABORATORIES	N01CM53817	150,000
A.P.	SIMONSEN LABORATORIES	N01CM53847	41,250

ANALYSIS OF CONTRACTS BY ACTIVITY

ACTIVITY

FOR FISCAL YEAR 1977
AS OF 05/31/77

AREA	CONTRACT	#	DOLLAR LEVEL
	STAGE II VERIFICATION SCREEN:		
A.P.	SOUTHERN ANIMAL FARMS	N01CM50599	54,390
A.P.	SOUTHERN ANIMAL FARMS	N01CM53845	31,250
D.T.P.	SOUTHERN RESEARCH INSTITUTE	N01CM43756	594,880
D.T.P.	SOUTHERN RESEARCH INSTITUTE	N01CM43784	300,603
A.P.	SPARTAN RESEARCH ANIMALS, INC.	N01CM50582	27,300
A.P.	TACONIC FARMS	N01CM50597	44,200
D.T.P.	TEXAS, UNIVERSITY OF, SYSTEM CANCER CTR/MDA	N01CM43786	64,170
D.T.P.	TEXAS, UNIVERSITY OF	N01CM53767	84,357
D.T.P.	UPJOHN COMPANY	N01CM43753	44,023
D.T.P.	VALUE ENGINEERING COMPANY	N01CM67086	187,248
D.T.P.	WARF INSTITUTE, INC.	N01CM53770	62,723
D.T.P.	WASHINGTON UNIVERSITY SCHOOL OF MEDICINE	N01CM43731	42,091
	TOTAL		6,693,022

ANALYSIS OF CONTRACTS BY ACTIVITY

ACTIVITY FOR FISCAL YEAR 1977
AS OF 05/31/77

AREA	CONTRACT	#	DOLLAR LEVEL
	STAGE II PROCUREMENT OF PRECLINICAL MATERIAL:		
D.T.P.	AGRICULTURE, DEPARTMENT OF	Y01CM40001	41,200
D.T.P.	ALDRICH CHEMICAL COMPANY, INC.	N01CM23706	143,553
O.D.	ARMY, DEPARTMENT OF, FT. DETRICK	Y01CO60603	36,297
D.T.P.	ASH STEVENS, INC.	N01CM57012	170,765
D.T.P.	ASSOCIATED BIOMEDIC SYSTEMS, INC.	N01CM02271	18,750
D.T.P.	BRITISH COLUMBIA, UNIVERSITY OF	N01CM23223	5,499
D.T.P.	COLLABORATIVE RESEARCH, INC.	N01CM77071	9,427
D.T.P.	CORDOVA CHEMICAL COMPANY	N01CM57031	25,150
D.T.P.	DOW CHEMICAL COMPANY	N01CM53768	22,500
D.T.P.	FARMITALIA	N01CM57014	19,316
D.T.P.	FARMITALIA	N01CM57036	18,959
D.T.P.	HEM RESEARCH	N01CM67066	53,350
D.T.P.	ILLINOIS, UNIVERSITY OF	N01CM67090	16,965
D.T.P.	LITTON BIONETICS, INC.	N01CM12340	424,993
D.T.P.	LITTON BIONETICS, INC.	N01CM77134	365,562
O.D.	LITTON BIONETICS, INC.	N01CO25423	500,000
D.T.P.	MICROBIOLOGICAL ASSOCIATES, INC.	N01CM43793	139,200
D.T.P.	MIDWEST RESEARCH INSTITUTE	N01CM33722	45,765
D.T.P.	MIDWEST RESEARCH INSTITUTE	N01CM33743	55,351
D.T.P.	MONSANTO RESEARCH CORPORATION	N01CM67046	5,850
D.T.P.	NORTHEASTERN UNIVERSITY	N01CM53741	3,618
D.T.P.	OHIO STATE UNIVERSITY RESEARCH FOUNDATION	N01CM57015	6,822
D.T.P.	PARKE, DAVIS AND COMPANY	N01CM23708	94,693
D.T.P.	PHARM-ECO	N01CM23707	42,328

ANALYSIS OF CONTRACTS BY ACTIVITY

FOR FISCAL YEAR 1977
AS OF 05/31/77

AREA	ACTIVITY CONTRACT	#	DOLLAR LEVEL
	STAGE II PROCUREMENT OF PRECLINICAL MATERIAL:		
D.T.P.	PURDUE UNIVERSITY	N01CM67091	18,866
D.T.P.	RESEARCH TRIANGLE INSTITUTE	N01CM67089	28,526
D.T.P.	RESEARCH TRIANGLE INSTITUTE	N01CM67121	188,000
D.T.P.	SLOAN-KETTERING INSTITUTE FOR CANCER RESEARCH	N01CM53820	20,592
D.T.P.	SOUTHERN RESEARCH INSTITUTE	N01CM43762	138,398
D.T.P.	STANFORD RESEARCH INSTITUTE	N01CM33723	72,695
D.T.P.	STANFORD RESEARCH INSTITUTE	N01CM83742	106,372
D.T.P.	STANFORD RESEARCH INSTITUTE	N01CM63776	191,280
D.T.P.	STARKS ASSOCIATES, INC.	N01CM23203	139,732
D.T.P.	STARKS ASSOCIATES, INC.	N01CM53769	130,823
D.T.P.	VETERANS ADMINISTRATION HOSPITAL	Y01CM60102	5,010
D.T.P.	VIRGINIA, UNIVERSITY OF	N01CM67002	23,694
	TOTAL		3,330,401

ANALYSIS OF CONTRACTS BY ACTIVITY

ACTIVITY FOR FISCAL YEAR 1977
AS OF 05/31/77

AREA	CONTRACT	#	DOLLAR LEVEL
	STAGE III PHARMACOLOGY/TOXICOLOGY:		
D.T.P.	ARTHUR D. LITTLE, INC.	N01CM53849	615,598
D.T.P.	BRITISH COLUMBIA, UNIVERSITY OF	N01CM23223	16,498
D.T.P.	COLORADO STATE UNIVERSITY	N01CM12192	10,772
D.T.P.	DOW CHEMICAL COMPANY	N01CM23712	172,718
CTEP	GEORGETOWN UNIVERSITY	N01CM67110	21,802
D.T.P.	HAZLETON LABORATORIES, INC.	N01CM33708	160,002
A.P.	HAZLETON LABORATORIES, INC.	N01CM60125	37,500
D.T.P.	HEALTH RESEARCH, INC.	N01CM33726	9,047
A.P.	LABORATORY RESEARCH ENTERPRISES, INC.	N01CM60124	36,875
D.T.P.	MARIO NEGRI INST OF PHARMACOLOGICAL RESEARCH	N01CM67064	75,580
A.P.	MARSHALL RESEARCH ANIMALS, INC.	N01CM60123	36,250
D.T.P.	OHIO STATE UNIVERSITY RESEARCH FOUNDATION	N01CM53828	11,726
D.T.P.	OREGON STATE UNIVERSITY	N01CM23201	92,840
BCRC	PUBLIC HEALTH SERVICE	Y01CM40003	48,935
D.T.P.	SOUTHERN CALIFORNIA, UNIVERSITY OF	N01CM67065	97,500
D.T.P.	SOUTHERN RESEARCH INSTITUTE	N01CM43756	45,760
D.T.P.	SOUTHERN RESEARCH INSTITUTE	N01CM43780	215,000
D.T.P.	SOUTHERN RESEARCH INSTITUTE	N01CM57000	236,103
D.T.P.	TEXAS, UNIVERSITY OF, M.D. ANDERSON	N01CM53773	338,163
D.T.P.	UPJOHN COMPANY	N01CM43753	66,034
D.T.P.	VETERANS ADMINISTRATION	Y01CM60103	554
D.T.P.	YALE UNIVERSITY SCHOOL OF MEDICINE	N01CM67068	17,500
		TOTAL	2,362,757

ANALYSIS OF CONTRACTS BY ACTIVITY

ACTIVITY

FOR FISCAL YEAR 1977
AS OF 05/31/77

AREA	CONTRACT	#	DOLLAR LEVEL
	STAGE III PROD. AND FORM. FOR CLINICAL TRIALS:		
D.T.P.	ALDRICH CHEMICAL COMPANY, INC.	N01CM23706	61,523
D.T.P.	ASH STEVENS, INC.	N01CM57012	56,922
D.T.P.	BEN VENUE LABORATORIES, INC.	N01CM67109	970,083
D.T.P.	CORDOVA CHEMICAL COMPANY	N01CM57031	75,450
D.T.P.	DOW CHEMICAL COMPANY	N01CM53768	67,500
D.T.P.	ELKINS-SINN, INC.	N01CM67103	117,786
D.T.P.	FLOW LABORATORIES, INC.	N01CM67088	122,180
D.T.P.	IOWA, UNIVERSITY OF	N01CM43743	154,802
C.O.P.	LITTON BIONETICS, INC.	N01CM67067	31,178
D.T.P.	MICROBIOLOGICAL ASSOCIATES, INC.	N01CM43793	46,400
D.T.P.	MIDWEST RESEARCH INSTITUTE	N01CM33722	183,058
D.T.P.	MONSANTO RESEARCH CORPORATION	N01CM67046	52,650
D.T.P.	MONSANTO RESEARCH CORPORATION	N01CM77140	478,137
D.T.P.	PARKE, DAVIS AND COMPANY	N01CM23708	94,693
D.T.P.	PHARM-ECO	N01CM23707	98,765
D.T.P.	PHILIPS ROXANE LABORATORIES, INC.	N01CM67053	101,537
D.T.P.	RESEARCH TRIANGLE INSTITUTE	N01CM67121	47,000
D.T.P.	STANFORD RESEARCH INSTITUTE	N01CM33723	290,779
D.T.P.	STANFORD RESEARCH INSTITUTE	N01CM63776	47,820
D.T.P.	STARKS ASSOCIATES, INC.	N01CM23203	139,732
D.T.P.	STARKS ASSOCIATES, INC.	N01CM53769	43,608
		TOTAL	3,281,603

ANALYSIS OF CONTRACTS BY ACTIVITY

ACTIVITY FOR FISCAL YEAR 1977
AS OF 05/31/77

AREA	CONTRACT	#	DOLLAR LEVEL
	PRECLIN. BASIC RES. - BIOLOGICAL STUDIES:		
D.T.P.	ARIZONA, UNIVERSITY OF	N01CM33713	8,178
D.T.P.	ARTHUR D. LITTLE, INC.	N01CM53849	119,922
O.D.	ARTHUR D. LITTLE, INC.	N01CM63823	185,814
D.T.P.	ASSOCIATED BIOMEDIC SYSTEMS, INC.	N01CM02271	18,750
D.T.P.	BRIGHAM YOUNG UNIVERSITY	N01CM43790	18,230
D.T.P.	COLORADO, UNIVERSITY OF, MEDICAL CENTER	N01CM12186	87,500
O.D.	ENERGY RES & DEV ADM	Y01CM40102	10,500
D.T.P.	HARVARD COLLEGE	N01CM53825	36,297
D.T.P.	HAZLETON LABORATORIES, INC.	N01CM33708	120,002
C.O.P.	HAZLETON LABORATORIES, INC.	N01CM43702	67,910
C.O.P.	HAZLETON LABORATORIES, INC.	N01CM77126	30,027
D.T.P.	HEALTH RESEARCH, INC.	N01CM57039	104,515
D.T.P.	HEM RESEARCH	N01CM67066	53,350
D.T.P.	INSTITUT JULES BORDET	N01CM53840	18,780
D.T.P.	JOHNS HOPKINS UNIVERSITY	N01CM43718	43,050
D.T.P.	LITTON BIONETICS, INC.	N01CM12340	424,993
D.T.P.	LITTON BIONETICS, INC.	N01CM22062	257,926
C.O.P.	LITTON BIONETICS, INC.	N01CM67057	280,500
D.T.P.	LITTON BIONETICS, INC.	N01CM77134	365,562
D.T.P.	MIAMI, UNIVERSITY OF	N01CM57041	106,157
C.O.P.	MICROBIOLOGICAL ASSOCIATES, INC.	N01CM63810	200,000
O.D.	NAVAL WEAPONS CENTER	Y01CM50101	34,000
BCRC	PUBLIC HEALTH SERVICE	Y01CM40003	76,398
D.T.P.	RES. FDN. OF STATE UNIVERSITY OF NEW YORK	N01CM53766	151,164

ANALYSIS OF CONTRACTS BY ACTIVITY

AREA	ACTIVITY CONTRACT	FOR FISCAL YEAR 1977 AS OF 05/31/77 #	DOLLAR LEVEL
	PRECLIN. BASIC RES. - BIOLOGICAL STUDIES:		
D.T.P.	SLOAN-KETTERING INSTITUTE FOR CANCER RESEARCH	N01CM53820	61,775
D.T.P.	SMALL BUSINESS ADMINISTRATION	N01CM43719	45,146
D.T.P.	SOUTHERN RESEARCH INSTITUTE	N01CM43756	22,880
CTEP	WALTER REED ARMY MEDICAL CENTER	Y01CM40005	62,099
	TOTAL		3,012,025

ANALYSIS OF CONTRACTS BY ACTIVITY

ACTIVITY

FOR FISCAL YEAR 1977
AS OF 05/31/77

AREA	CONTRACT	#	DOLLAR LEVEL
	PRECLIN. BASIC RES. - TREATMENT STUDIES:		
O.D.	ARMY RES. INST. OF ENVIRONMENTAL MEDICINE	Y01CM60105	24,500
D.T.P.	ARTHUR D. LITTLE, INC.	N01CM53765	367,214
D.T.P.	BATTELLE MEMORIAL INSTITUTE	N01CM57005	38,799
D.T.P.	CALIFORNIA, UNIVERSITY OF	N01CM43791	34,804
D.T.P.	CATHOLIC MEDICAL CTR OF BROOKLYN AND QUEENS	N01CM67081	102,920
C.O.P.	HAZLETON LABORATORIES, INC.	N01CM43702	67,910
C.O.P.	HAZLETON LABORATORIES, INC.	N01CM77126	12,869
C.O.P.	HAZLETON RESEARCH ANIMALS, INC.	N01CM77144	24,822
D.T.P.	IIT RESEARCH INSTITUTE	N01CM57004	25,952
D.T.P.	MARIO NEGRI INST OF PHARMACOLOGICAL RESEARCH	N01CM53826	96,809
D.T.P.	MASON RESEARCH INSTITUTE	N01CM57003	69,682
C.O.P.	MASON RESEARCH INSTITUTE	N01CM67011	189,907
D.T.P.	RADIOBIOLOGICAL INST., ORGAN. FOR HEALTH RES.	N01CM53763	73,151
D.T.P.	SOUTHERN RESEARCH INSTITUTE	N01CM43756	869,440
D.T.P.	SOUTHERN RESEARCH INSTITUTE	N01CM57000	101,187
D.T.P.	VALUE ENGINEERING COMPANY	N01CM67086	74,899
D.T.P.	WARF INSTITUTE, INC.	N01CM53770	6,969
D.T.P.	WASHINGTON UNIVERSITY SCHOOL OF MEDICINE	N01CM43731	42,091
	TOTAL		2,223,925

ANALYSIS OF CONTRACTS BY ACTIVITY

ACTIVITY

FOR FISCAL YEAR 1977
AS OF 05/31/77

AREA	CONTRACT	#	DOLLAR LEVEL
	PROGRAM MANAGEMENT:		
D.T.P.	ARTHUR D. LITTLE, INC.	N01CM33727	180,000
O.D.	ARTHUR D. LITTLE, INC.	N01CM63823	44,955
BCRC	BOWMAN GRAY SCHOOL OF MEDICINE	N01CM67054	3,989
BCRC	CALIFORNIA, UNIVERSITY OF	N01CM53752	13,362
BCRC	CALIFORNIA, UNIVERSITY OF	N01CM67055	7,693
BCRC	CLINICA NEUROCHIRURGICA DELL UNIVERSITA	N01CM67056	2,227
BCRC	CONTROL DATA CORPORATION	N01CM67107	5,000
O.D.	DOT SYSTEMS, INC.	N01CM53774	79,617
BCRC	DUKE UNIVERSITY	N01CM67010	9,853
O.D.	FRANKLIN INSTITUTE RESEARCH LABS.	N01CM77092	401,310
BCRC	HAZLETON LABORATORIES, INC.	N01CM67080	12,767
BCRC	INDIANA UNIVERSITY FOUNDATION	N01CM67057	3,370
CTEP	INFORMATION PLANNING ASSOC.	N01CM77104	90,195
D.T.P.	INSTITUT JULES BORDET	N01CM53840	46,950
D.T.P.	JAPANESE FOUNDATION FOR CANCER RESEARCH	N01CM22054	14,100
BCRC	KENTUCKY, UNIVERSITY OF	N01CM67058	4,536
BCRC	MARYLAND, UNIVERSITY OF	N01CM43748	204,086
BCRC	MEMORIAL HOSP. FOR CANCER & ALLIED DISEASES	N01CM57019	13,939
BCRC	MICROBIOLOGICAL ASSOCIATES, INC.	N01CM77131	6,503
BCRC	MONTEFIORE HOSPITAL	N01CM67061	8,829
A.P.	NATIONAL ACADEMY OF SCIENCES	N01CM53850	20,000
A.P.	NATIONAL ACADEMY OF SCIENCES	N01CM57013	24,000
BCRC	NEW YORK UNIVERSITY MEDICAL CENTER	N01CM67059	7,246
BCRC	OHIO STATE UNIVERSITY RESEARCH FOUNDATION	N01CM67060	2,812

ANALYSIS OF CONTRACTS BY ACTIVITY

ACTIVITY FOR FISCAL YEAR 1977
AS OF 05/31/77

AREA	CONTRACT	#	DOLLAR LEVEL
	PROGRAM MANAGEMENT:		
BCRC	PUBLIC HEALTH SERVICE	Y01CM40003	13,982
BCRC	SAINT LOUIS UNIVERSITY SCHOOL OF MEDICINE	N01CM57020	12,255
BCRC	TENNESSEE, UNIVERSITY OF	N01CM57021	9,980
D.T.P.	VALUE ENGINEERING COMPANY	N01CM53821	125,000
BCRC	VERMONT, UNIVERSITY OF, COLLEGE OF MEDICINE	N01CM33716	8,000
	TOTAL		1,376,556

ANALYSIS OF CONTRACTS BY ACTIVITY

ACTIVITY

FOR FISCAL YEAR 1977
AS OF 05/31/77

AREA	CONTRACT	#	DOLLAR LEVEL
	PHASE I CLINICAL TRIALS:		
O.D.	ARTHUR D. LITTLE, INC.	N01CM63823	35,964
CTEP	CHILDREN'S HOSPITAL OF LOS ANGELES	N01CM43803	98,173
CTEP	GEORGETOWN UNIVERSITY	N01CM67110	32,703
D.T.P.	JAPANESE FOUNDATION FOR CANCER RESEARCH	N01CM22054	10,575
CTEP	KANSAS, UNIVERSITY OF	N01CM43802	84,652
BCRC	MARYLAND, UNIVERSITY OF	N01CM43748	408,173
CTEP	MAYO FOUNDATION	N01CM53838	64,234
CTEP	MEMORIAL HOSP. FOR CANCER & ALLIED DISEASES	N01CM43804	140,408
CTEP	MOUNT SINAI SCHOOL OF MEDICINE	N01CM53837	93,752
D.T.P.	OHIO STATE UNIVERSITY RESEARCH FOUNDATION	N01CM53828	93,806
CTEP	SIDNEY FARBER CANCER INSTITUTE	N01CM53839	88,983
CTEP	TEXAS, UNIVERSITY OF, SYSTEM CANCER CTR/MDA	N01CM43801	166,572
D.T.P.	TEXAS, UNIVERSITY OF, M.D. ANDERSON	N01CM53773	225,442
D.T.P.	YALE UNIVERSITY SCHOOL OF MEDICINE	N01CM67068	17,500
		TOTAL	1,561,037

ANALYSIS OF CONTRACTS BY ACTIVITY

ACTIVITY

FOR FISCAL YEAR 1977
AS OF 05/31/77

AREA	CONTRACT	#	DOLLAR LEVEL
	PHASE II CLINICAL TRIALS:		
BCRC	BOWMAN GRAY SCHOOL OF MEDICINE	N01CM67054	11,966
BCRC	CALIFORNIA, UNIVERSITY OF	N01CM53752	40,085
BCRC	CALIFORNIA, UNIVERSITY OF	N01CM67055	23,080
CTEP	CALIFORNIA, UNIVERSITY OF	N01CM77151	21,801
BCRC	CLINICA NEUROCHIRURGICA DELL UNIVERSITA	N01CM67056	6,680
BCRC	DUKE UNIVERSITY	N01CM67010	29,559
CTEP	FRED HUTCHINSON CANCER RESEARCH CENTER	N01CM77148	23,163
CTEP	GEORGETOWN UNIVERSITY	N01CM67110	32,703
CTEP	HEALTH RESEARCH, INC.	N01CM43794	10,445
CTEP	HEALTH RESEARCH, INC.	N01CM53834	52,852
BCRC	INDIANA UNIVERSITY FOUNDATION	N01CM67057	10,109
CTEP	IST. NAZION PER LO STUD E LA CURA DEI TUMORI	N01CM33714	21,760
CTEP	IST. NAZION PER LO STUD E LA CURA DEI TUMORI	N01CM43726	38,150
D.T.P.	JAPANESE FOUNDATION FOR CANCER RESEARCH	N01CM22054	10,575
BCRC	KENTUCKY, UNIVERSITY OF	N01CM67058	13,608
CTEP	LEEDS, UNIVERSITY OF	N01CM43727	16,450
BCRC	MARYLAND, UNIVERSITY OF	N01CM43748	1,387,788
CTEP	MAYO FOUNDATION	N01CM02066	131,250
CTEP	MAYO FOUNDATION	N01CM43796	63,328
CTEP	MAYO FOUNDATION	N01CM53772	121,385
CTEP	MAYO FOUNDATION	N01CM57044	279,366
CTEP	MAYO FOUNDATION	N01CM77150	35,128
CTEP	MEMORIAL HOSP. FOR CANCER & ALLIED DISEASES	N01CM53844	18,099
BCRC	MEMORIAL HOSP. FOR CANCER & ALLIED DISEASES	N01CM57019	41,816

ANALYSIS OF CONTRACTS BY ACTIVITY

ACTIVITY

FOR FISCAL YEAR 1977
AS OF 05/31/77

AREA	CONTRACT	#	DOLLAR LEVEL
	PHASE II CLINICAL TRIALS:		
CTEP	MEMORIAL HOSP. FOR CANCER & ALLIED DISEASES	N01CM57043	262,843
CTEP	MIAMI, UNIVERSITY OF	N01CM43800	57,092
BCRC	MONTEFIORE HOSPITAL	N01CM67061	26,487
BCRC	NEW YORK UNIVERSITY MEDICAL CENTER	N01CM67059	21,739
CTEP	OHIO STATE UNIVERSITY RESEARCH FOUNDATION	N01CM33736	6,379
D.T.P.	OHIO STATE UNIVERSITY RESEARCH FOUNDATION	N01CM53828	11,726
BCRC	OHIO STATE UNIVERSITY RESEARCH FOUNDATION	N01CM67050	8,437
CTEP	ONTARIO CANCER INSTITUTE	N01CM77152	29,353
BCRC	SAINT LOUIS UNIVERSITY SCHOOL OF MEDICINE	N01CM57020	36,764
CTEP	SIDNEY FARBER CANCER INSTITUTE	N01CM67037	223,722
BCRC	TENNESSEE, UNIVERSITY OF	N01CM57021	29,939
CTEP	TEXAS, UNIVERSITY OF, SYSTEM CANCER CTR/MDA	N01CM57042	340,408
CTEP	TEXAS, UNIVERSITY OF, M.D. ANDERSON	N01CM77149	28,030
CTEP	TEXAS, UNIVERSITY OF, SYSTEM CANCER CTR/MDA	N01CM77153	24,594
CTEP	VANDERBILT UNIVERSITY MEDICAL CENTER	N01CM77122	21,687
CTEP	WAYNE STATE UNIVERSITY	N01CM67105	244,566
CTEP	YALE UNIVERSITY SCHOOL OF MEDICINE	N01CM43797	39,354
	TOTAL		3,854,266

ANALYSIS OF CONTRACTS BY ACTIVITY

ACTIVITY FOR FISCAL YEAR 1977
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AREA	CONTRACT	#	DOLLAR LEVEL
	PHASE III CLINICAL TRIALS:		
BCRC	BOWMAN GRAY SCHOOL OF MEDICINE	N01CM67054	23,931
BCRC	CALIFORNIA, UNIVERSITY OF	N01CM53752	80,171
BCRC	CALIFORNIA, UNIVERSITY OF	N01CM67055	46,160
CTEP	CALIFORNIA, UNIVERSITY OF	N01CM77151	65,402
BCRC	CLINICA NEUROCHIRURGICA DELL UNIVERSITA	N01CM67056	13,360
BCRC	DUKE UNIVERSITY	N01CM67010	59,117
CTEP	FRED HUTCHINSON CANCER RESEARCH CENTER	N01CM77148	69,489
CTEP	GEORGETOWN UNIVERSITY	N01CM67110	65,406
CTEP	HEALTH RESEARCH, INC.	N01CM67117	62,088
C.O.P.	HEBREW UNIVERSITY	N01CM12127	50,000
BCRC	INDIANA UNIVERSITY FOUNDATION	N01CM67057	20,218
CTEP	IST. NAZION PER LO STUD E LA CURA DEI TUMORI	N01CM33714	50,774
BCRC	KENTUCKY, UNIVERSITY OF	N01CM67058	27,216
C.O.P.	MAKERERE UNIVERSITY COLLEGE COUNCIL	N01CM71343	67,252
BCRC	MARYLAND, UNIVERSITY OF	N01CM43748	1,061,249
CTEP	MAYO FOUNDATION	N01CM02066	43,750
CTEP	MAYO FOUNDATION	N01CM43783	52,856
CTEP	MAYO FOUNDATION	N01CM57044	228,572
CTEP	MAYO FOUNDATION	N01CM67123	107,126
CTEP	MAYO FOUNDATION	N01CM77150	105,384
CTEP	MEMORIAL HOSP. FOR CANCER & ALLIED DISEASES	N01CM53844	95,017
BCRC	MEMORIAL HOSP. FOR CANCER & ALLIED DISEASES	N01CM57019	83,632
CTEP	MEMORIAL HOSP. FOR CANCER & ALLIED DISEASES	N01CM57043	175,229
CTEP	MIAMI, UNIVERSITY OF	N01CM43800	89,715

ANALYSIS OF CONTRACTS BY ACTIVITY

ACTIVITY FOR FISCAL YEAR 1977
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AREA	CONTRACT	#	DOLLAR LEVEL
	PHASE III CLINICAL TRIALS:		
BCRC	MONTEFIORE HOSPITAL	N01CM67061	52,973
CTEP	MOUNT SINAI SCHOOL OF MEDICINE	N01CM43703	49,492
CTEP	NATIONAL NAVAL MEDICAL CENTER	Y01CM50100	31,500
BCRC	NEW YORK UNIVERSITY MEDICAL CENTER	N01CM67059	43,477
CTEP	OHIO STATE UNIVERSITY RESEARCH FOUNDATION	N01CM33736	19,137
BCRC	OHIO STATE UNIVERSITY RESEARCH FOUNDATION	N01CM67060	16,874
CTEP	ONTARIO CANCER INSTITUTE	N01CM77152	88,060
BCRC	SAINT LOUIS UNIVERSITY SCHOOL OF MEDICINE	N01CM57020	73,528
CTEP	SIDNEY FARBER CANCER INSTITUTE	N01CM67037	183,046
BCRC	TENNESSEE, UNIVERSITY OF	N01CM57021	59,878
CTEP	TEXAS, UNIVERSITY OF, M.D. ANDERSON	N01CM33710	184,931
CTEP	TEXAS, UNIVERSITY OF, SYSTEM CANCER CTR/MDA	N01CM57042	278,515
CTEP	TEXAS, UNIVERSITY OF, SYSTEM CANCER CTR/MDA	N01CM67026	26,302
CTEP	TEXAS, UNIVERSITY OF, SYSTEM CANCER CTR/MDA	N01CM67116	42,884
CTEP	TEXAS, UNIVERSITY OF, M.D. ANDERSON	N01CM77149	84,089
CTEP	TEXAS, UNIVERSITY OF, SYSTEM CANCER CTR/MDA	N01CM77153	73,781
CTEP	VANDERBILT UNIVERSITY MEDICAL CENTER	N01CM77122	65,060
BCRC	VERMONT, UNIVERSITY OF, COLLEGE OF MEDICINE	N01CM33716	32,000
C.O.P.	VETERANS ADMINISTRATION	Y01CM70106	862,800
CTEP	VETERANS ADMINISTRATION	Y01CM70107	550,400
CTEP	WAYNE STATE UNIVERSITY	N01CM67105	200,100
		TOTAL	5,762,041

ANALYSIS OF CONTRACTS BY ACTIVITY

ACTIVITY

FOR FISCAL YEAR 1977
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AREA	CONTRACT	#	DOLLAR LEVEL
	PHASE IV CLINICAL TRIALS (COMBINED MODALITY):		
CTEP	ALBANY MEDICAL COLLEGE OF UNION UNIVERSITY	N01CM57032	125,099
CTEP	CALIFORNIA, UNIVERSITY OF	N01CM67097	155,216
CTEP	CALIFORNIA, UNIVERSITY OF	N01CM77151	21,801
CTEP	CHICAGO, UNIVERSITY OF	N01CM67095	191,315
CTEP	FRED HUTCHINSON CANCER RESEARCH CENTER	N01CM77148	23,163
CTEP	GEORGETOWN UNIVERSITY	N01CM67094	88,609
CTEP	HEALTH RESEARCH, INC.	N01CM43782	86,527
CTEP	HEALTH RESEARCH, INC.	N01CM43794	17,409
CTEP	HEALTH RESEARCH, INC.	N01CM57034	137,023
CTEP	HEALTH RESEARCH, INC.	N01CM67117	62,088
CTEP	IST. NAZION PER LO STUD E LA CURA DEI TUMORI	N01CM33714	72,535
CTEP	IST. NAZION PER LO STUD E LA CURA DEI TUMORI	N01CM43726	57,225
BCRC	MARYLAND, UNIVERSITY OF	N01CM43748	1,020,432
CTEP	MAYO FOUNDATION	N01CM43783	59,463
CTEP	MAYO FOUNDATION	N01CM43796	105,547
CTEP	MAYO FOUNDATION	N01CM57033	162,872
CTEP	MAYO FOUNDATION	N01CM67123	107,126
CTEP	MAYO FOUNDATION	N01CM77150	35,128
CTEP	MIAMI, UNIVERSITY OF	N01CM43800	16,312
CTEP	MIAMI, UNIVERSITY OF	N01CM67093	106,843
CTEP	MOUNT SINAI SCHOOL OF MEDICINE	N01CM53841	172,824
CTEP	MOUNT SINAI SCHOOL OF MEDICINE	N01CM67096	108,221
CTEP	OHIO STATE UNIVERSITY RESEARCH FOUNDATION	N01CM33736	6,379
CTEP	ONTARIO CANCER INSTITUTE	N01CM77152	29,353

ANALYSIS OF CONTRACTS BY ACTIVITY

ACTIVITY

FOR FISCAL YEAR 1977
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AREA	CONTRACT	#	DOLLAR LEVEL
	PHASE IV CLINICAL TRIALS (COMBINED MODALITY):		
CTEP	SIDNEY FARBER CANCER INSTITUTE	N01CM43781	18,672
CTEP	SIDNEY FARBER CANCER INSTITUTE	N01CM57035	145,942
CTEP	SOUTHERN CALIFORNIA, UNIVERSITY OF	N01CM53842	18,077
CTEP	TEXAS, UNIVERSITY OF, SYSTEM CANCER CTR/MDA	N01CM67026	26,302
CTEP	TEXAS, UNIVERSITY OF, SYSTEM CANCER CTR/MDA	N01CM67116	42,884
CTEP	TEXAS, UNIVERSITY OF, M.D. ANDERSON	N01CM77149	28,030
CTEP	TEXAS, UNIVERSITY OF, SYSTEM CANCER CTR/MDA	N01CM77153	24,594
CTEP	VANDERBILT UNIVERSITY MEDICAL CENTER	N01CM77122	21,687
C.O.P.	VETERANS ADMINISTRATION	Y01CM70106	862,800
CTEP	VETERANS ADMINISTRATION	Y01CM70107	550,400
CTEP	YALE UNIVERSITY SCHOOL OF MEDICINE	N01CM43797	65,590
CTEP	YALE UNIVERSITY SCHOOL OF MEDICINE	N01CM53843	86,804
	TOTAL		4,860,292

ANALYSIS OF CONTRACTS BY ACTIVITY

ACTIVITY

FOR FISCAL YEAR 1977
AS OF 05/31/77

AREA	CONTRACT	#	DOLLAR LEVEL
	CLINICAL TRIALS - OTHER RESEARCH:		
BCRC	CONTROL DATA CORPORATION	N01CM67107	45,000
CTEP	HEALTH RESEARCH, INC.	N01CM43794	6,963
CTEP	MAYO FOUNDATION	N01CM43783	19,821
CTEP	MAYO FOUNDATION	N01CM43796	42,219
CTEP	MAYO FOUNDATION	N01CM53838	16,058
CTEP	MAYO FOUNDATION	N01CM57033	40,718
CTEP	MOUNT SINAI SCHOOL OF MEDICINE	N01CM53837	23,438
C.O.P.	VETERANS ADMINISTRATION	Y01CM70106	431,400
CTEP	VETERANS ADMINISTRATION	Y01CM70107	275,200
CTEP	YALE UNIVERSITY SCHOOL OF MEDICINE	N01CM43797	26,236
	TOTAL		927,053

ANALYSIS OF CONTRACTS BY ACTIVITY

ACTIVITY FOR FISCAL YEAR 1977
AS OF 05/31/77

AREA	CONTRACT	#	DOLLAR LEVEL
	CLINICAL TRIALS SUPPORTIVE RESEARCH:		
C.O.P.	ABBOTT LABORATORIES	N01CM43704	49,733
D.T.P.	ARIZONA, UNIVERSITY OF	N01CM33713	32,710
C.O.P.	CALIFORNIA, UNIVERSITY OF	N01CM02092	400,000
O.D.	CALIFORNIA, UNIVERSITY OF	N01HB62971	151,313
C.O.P.	CHILDREN'S HOSPITAL OF LOS ANGELES	N01CM53831	24,402
CTEP	CHILDREN'S HOSPITAL MEDICAL CENTER	N01CP65826	34,372
CTEP	CHILDREN'S HOSPITAL OF LOS ANGELES	N01CP65828	62,749
D.T.P.	ENERGY RES & DEV ADM	Y01CM10060	35,410
O.D.	ENERGY RES & DEV ADM	Y01CM40101	12,800
O.D.	ENERGY RES & DEV ADM	Y01CM40102	94,500
CTEP	FRONTIER SCIENCE & TECHNOLOGY RES. FDN., INC.	N01CM77136	170,991
CTEP	GEORGETOWN UNIVERSITY	N01CM33745	175,314
CTEP	GEORGETOWN UNIVERSITY	N01CM67110	65,406
BCRC	HAZLETON LABORATORIES, INC.	N01CM67080	412,807
CTEP	HEALTH RESEARCH, INC.	N01CM43782	9,614
C.O.P.	INSTITUTO NAZIONALE LA CURA DEI TOMORI	N01CM67115	21,715
O.D.	IOWA, UNIVERSTIY OF	N01HB62973	135,364
D.T.P.	KANSAS, UNIVERSITY OF	N01CM12184	56,500
D.T.P.	KENTUCKY, UNIVERSITY OF	N01CM67038	159,075
CTEP	LEEDS, UNIVERSITY OF	N01CM43727	16,450
CTEP	MEMORIAL HOSP. FOR CANCER & ALLIED DISEASES	N01CP65829	31,979
BCRC	MICROBIOLOGICAL ASSOCIATES, INC.	N01CM77131	86,397
C.O.P.	MINNESOTA, UNIVERSITY OF	N01CM67113	54,640
O.D.	MINNESOTA, UNIVERSITY OF	N01HB62972	134,528

ANALYSIS OF CONTRACTS BY ACTIVITY

ACTIVITY FOR FISCAL YEAR 1977
AS OF 05/31/77

AREA	CONTRACT	#	DOLLAR LEVEL
	CLINICAL TRIALS SUPPORTIVE RESEARCH:		
D.T.P.	MISSOURI, UNIVERSITY OF	N01CM12323	210,000
CTEP	MOUNT SINAI SCHOOL OF MEDICINE	N01CM53841	19,203
CTEP	MOUNT SINAI SCHOOL OF MEDICINE	N01CM67096	12,025
C.O.P.	NEW ENGLAND MEDICAL CENTER	N01CM67112	62,679
CTEP	RES. FDN. OF STATE UNIVERSITY OF NEW YORK	N01CM33715	192,424
CTEP	SOUTHERN CALIFORNIA, UNIVERSITY OF	N01CM53842	2,009
D.T.P.	SOUTHERN CALIFORNIA, UNIVERSITY OF	N01CM67065	32,500
CTEP	STANFORD UNIVERSITY	N01CP65825	44,100
C.O.P.	STANFORD UNIVERSITY, BOARD OF TRUSTEES	N01CM67072	90,000
C.O.P.	STANFORD UNIVERSITY, BOARD OF TRUSTEES	N01CM67111	84,000
C.O.P.	TEXAS, UNIVERSITY OF	N01CM53832	628,793
CTEP	TEXAS, UNIVERSITY OF, SYSTEM CANCER CTR/MDA	N01CP65794	35,096
O.D.	WASHINGTON UNIVERSITY	N01HB62970	121,511
CTEP	YALE UNIVERSITY SCHOOL OF MEDICINE	N01CM53843	9,645
		TOTAL	3,973,354
		GRAND TOTAL	68,200,830

TABLE VI

ANALYSIS OF DIVISION OF CANCER TREATMENT

ACTIVITIES BY
ACTIVE CONTRACTS

FOR FISCAL YEAR 1977
AS OF 05/31/77

NAME	# & AREA	PERCENT OF EFFORT		DOLLAR LEVEL
ABBOTT LABORATORIES	N01CM43704 C.O.P.			49,733
CLINICAL TRIALS SUPPORTIVE RESEARCH		*100.00*	49,733	
LEUK.-ACUTE GRAN. BLOOD PRODUCTS				
AGRICULTURE, DEPARTMENT OF	Y01CM40001 D.T.P.			412,000
ACQUISITION OF MATERIALS		* 90.00*	370,800	
NEW AGENT PROCUREMENT, PLANT PRODUCTS		85.00	350,200	
DATA PROCESSING AND SUPPORT		5.00	20,600	
PROCUREMENT OF PRECLINICAL MATERIAL		* 10.00*	41,200	
PLANT PRODUCTS				
ALABAMA, UNIVERSITY OF	N01CM57083 A.P.			105,520
ACQUISITION OF MATERIALS		* 20.00*	21,104	
ANIMALS				
BASIC SCREEN		* 55.00*	58,036	
ANIMALS				
VERIFICATION SCREEN		* 25.00*	26,380	
ANIMALS				
ALBANY MEDICAL COLLEGE OF UNION	N01CM57032 CTEP			125,099
PHASE IV CLINICAL TRIALS		*100.00*	125,099	
COLORECTAL SURG.		30.00	37,530	
COLORECTAL RAD.		10.00	12,510	
COLORECTAL CHEM.		25.00	31,275	
COLORECTAL IMM.		25.00	31,275	
COLORECTAL SUPPORTIVE CARE		10.00	12,510	
ALBERT EINSTEIN COLLEGE OF MEDICINE	N01CM43787 D.T.P.			74,172
VERIFICATION SCREEN		*100.00*	74,172	
DETAILED DRUG EVALUATION, IN VITRO		80.00	59,338	
DET. DRUG EVAL., RELATED NEW MODEL DEVEL		20.00	14,834	
ALDRICH CHEMICAL COMPANY, INC.	N01CM23706 D.T.P.			205,076
PROCUREMENT OF PRECLINICAL MATERIAL		* 70.00*	143,553	
SYNTHETICS				
PROD. AND FORM. FOR CLINICAL TRIALS		* 30.00*	61,523	
PRODUCTION, SYNTHETICS				

NAME	# & AREA	PERCENT OF EFFORT		DOLLAR LEVEL
ARIZONA STATE UNIVERSITY	N01CM67048 D.T.P.			125,019
ACQUISITION OF MATERIALS		*100.00*	125,019	
NEW AGENT PROCUREMENT, MARINE/ARTHROPODS				
ARIZONA, UNIVERSITY OF	N01CM33713 D.T.P.			40,888
BIOLOGICAL STUDIES		* 20.00*	8,178	
MARKERS				
CLINICAL TRIALS SUPPORTIVE RESEARCH		* 80.00*	32,710	
MYELOMA MARKERS		60.00	24,533	
MYELOMA DATA PROC. & SUPP.		20.00	8,178	
ARMY RES. INST. OF ENVIRONMENTAL	Y01CM60105 O.D.			24,500
TREATMENT STUDIES		*100.00*	24,500	
HYPERTHERMIA				
ARMY, DEPARTMENT OF, FT. DETRICK	Y01CO60603 O.D.			362,972
ACQUISITION OF MATERIALS		* 48.00*	174,227	
NEW AGENT PROCUREMENT, FERMENT/ANTIBIOT				
BASIC SCREEN		* 12.00*	43,557	
PRIMARY SCREENING, IN VITRO		3.00	10,889	
ANIMALS		9.00	32,667	
VERIFICATION SCREEN		* 30.00*	108,892	
ANIMALS				
PROCUREMENT OF PRECLINICAL MATERIAL		* 10.00*	36,297	
FERMENTATION/ANTIBIOTICS				
ARS/SPRAGUE-DAWLEY	N01CM53846 A.P.			175,000
ACQUISITION OF MATERIALS		* 20.00*	35,000	
ANIMALS				
BASIC SCREEN		* 55.00*	96,250	
ANIMALS				
VERIFICATION SCREEN		* 25.00*	43,750	
ANIMALS				
ARS/SPRAGUE-DAWLEY	N01CM53813 A.P.			1,000,000
ACQUISITION OF MATERIALS		* 20.00*	200,000	
ANIMALS				
BASIC SCREEN		* 55.00*	550,000	
ANIMALS				
VERIFICATION SCREEN		* 25.00*	250,000	
ANIMALS				

NAME	# & AREA	PERCENT OF EFFORT		DOLLAR LEVEL
ARTHUR D. LITTLE, INC.	N01CM67047 BCRC			129,913
VERIFICATION SCREEN		*100.00*	129,913	
DET. DRUG EVAL., RELATED NEW MODEL DEVEL				
ARTHUR D. LITTLE, INC.	N01CM53849 D.T.P.			799,478
BASIC SCREEN		* 8.00*	63,958	
TUMOR BANK				
PHARMACOLOGY/TOXICOLOGY		* 77.00*	615,598	
PHARMACOLOGY				
BIOLOGICAL STUDIES		* 15.00*	119,922	
BIOCHEMISTRY				
ARTHUR D. LITTLE, INC.	N01CM57006 D.T.P.			581,813
ACQUISITION OF MATERIALS		* 3.00*	17,454	
BIOASSAY OF NATURAL PRODUCTS				
BASIC SCREEN		* 72.00*	418,905	
PRIMARY SCREENING, IN VIVO		58.00	337,452	
PRIMARY SCREEN., RELATED NEW MODEL DEVEL		14.00	81,454	
VERIFICATION SCREEN		* 25.00*	145,453	
DETAILED DRUG EVALUATION, IN VIVO				
ARTHUR D. LITTLE, INC.	N01CM33727 D.T.P.			180,000
PROGRAM MANAGEMENT		*100.00*	180,000	
APP. MATH/DATA ANAL.				
ARTHUR D. LITTLE, INC.	N01CM53765 D.T.P.			816,031
BASIC SCREEN		* 55.00*	448,817	
IN VIVO ANALOG SCR.				
TREATMENT STUDIES		* 45.00*	367,214	
COMB. MODAL. THERAPY		45.00	367,214	
COMB. MODAL. THERAPY - RAD.		20.00	163,206	
COMB. MODAL. THERAPY - CHEM.		25.00	204,008	
ARTHUR D. LITTLE, INC.	N01CM67078 D.T.P.			215,399
ACQUISITION OF MATERIALS		* 5.00*	10,770	
BIOASSAY OF NATURAL PRODUCTS				
BASIC SCREEN		* 95.00*	204,629	
PRIMARY SCREENING, IN VITRO		90.00	193,859	
PRIMARY SCREEN., RELATED NEW MODEL DEVEL		5.00	10,770	
ARTHUR D. LITTLE, INC.	N01CM63823 O.D.			299,700
ACQUISITION OF MATERIALS		* 11.00*	32,967	
STRUCTURE ACTIVITY				

NAME	# & AREA	PERCENT OF EFFORT		DOLLAR LEVEL
BIOLOGICAL STUDIES		* 62.00*	185,814	
BLOOD PRODUCTS		12.00	35,964	
CELL KINETICS		15.00	44,955	
MOLECULAR BIOLOGY		20.00	59,940	
OTHER		15.00	44,955	
PROGRAM MANAGEMENT		* 15.00*	44,955	
ADMINISTRATION				
PHASE I CLINICAL TRIALS		* 12.00*	35,964	
PANCREATIC PHARM./TOX.				
ASH STEVENS, INC.	N01CM57012 D.T.P.			227,687
PROCUREMENT OF PRECLINICAL MATERIAL		* 75.00*	170,765	
SYNTHETICS				
PROD. AND FORM. FOR CLINICAL TRIALS		* 25.00*	56,922	
PRODUCTION, SYNTHETICS				
ASSOCIATED BIOMEDIC SYSTEMS, INC.	N01CM02271 D.T.P.			187,500
ACQUISITION OF MATERIALS		* 80.00*	150,000	
HUMAN BLOOD				
PROCUREMENT OF PRECLINICAL MATERIAL		* 10.00*	18,750	
ANALYTICAL AND QUALITY CONTROL				
BIOLOGICAL STUDIES		* 10.00*	18,750	
MOLECULAR BIOLOGY				
BATTELLE MEMORIAL INSTITUTE	N01CM57005 D.T.P.			775,981
ACQUISITION OF MATERIALS		* 15.00*	116,397	
BIOASSAY OF NATURAL PRODUCTS				
BASIC SCREEN		* 55.00*	426,790	
PRIMARY SCREENING, IN VIVO		50.00	387,991	
PRIMARY SCREEN., RELATED NEW MODEL DEVEL		5.00	38,799	
VERIFICATION SCREEN		* 25.00*	193,995	
DETAILED DRUG EVALUATION, IN VIVO				
TREATMENT STUDIES		* 5.00*	38,799	
CHEMOTHERAPY				
BATTELLE MEMORIAL INSTITUTE	N01CM67099 D.T.P.			454,410
BASIC SCREEN		*100.00*	454,410	
PRIMARY SCREENING, IN VIVO		75.00	340,808	
PRIMARY SCREEN., RELATED NEW MODEL DEVEL		25.00	113,603	
BAYLOR COLLEGE OF MEDICINE	N01CM57018 D.T.P.			3,040
VERIFICATION SCREEN		*100.00*	3,040	
DET. DRUG EVAL., RELATED NEW MODEL DEVEL				

NAME	# & AREA	PERCENT OF EFFORT		DOLLAR LEVEL
BELLAIRE ACRES	N01CM50583 A.P.			216,840
ACQUISITION OF MATERIALS		* 20.00*	43,368	
ANIMALS				
BASIC SCREEN		* 55.00*	119,262	
ANIMALS				
VERIFICATION SCREEN		* 25.00*	54,210	
ANIMALS				
BEN VENUE LABORATORIES, INC.	N01CM67109 D.T.P.			1,077,870
FORMULATION		* 10.00*	107,787	
DEVEL. OF EXP. FORMULATIONS				
PROD. AND FORM. FOR CLINICAL TRIALS		* 90.00*	970,083	
FORMULATION		75.00	808,403	
ANALYTICAL AND QUALITY CONTROL		15.00	161,681	
BIOTECH RESEARCH LABORATORIES, INC.	N01CM67052 D.T.P.			230,000
BASIC SCREEN		* 30.00*	69,000	
PRIMARY SCREENING, IN VITRO		10.00	23,000	
PRIMARY SCREEN., RELATED NEW MODEL DEVEL		20.00	46,000	
VERIFICATION SCREEN		* 70.00*	161,000	
DETAILED DRUG EVALUATION, IN VITRO		50.00	115,000	
DET. DRUG EVAL., RELATED NEW MODEL DEVEL		20.00	46,000	
BOWMAN GRAY SCHOOL OF MEDICINE	N01CM67054 BCRC			39,885
PROGRAM MANAGEMENT		* 10.00*	3,989	
ADMINISTRATION				
PHASE II CLINICAL TRIALS		* 30.00*	11,966	
CNS TUMORS CHEM.				
PHASE III CLINICAL TRIALS		* 60.00*	23,931	
CNS TUMORS RAD.		30.00	11,966	
CNS TUMORS CHEM.		30.00	11,966	
BRIGHAM YOUNG UNIVERSITY	N01CM43790 D.T.P.			60,765
VERIFICATION SCREEN		* 70.00*	42,536	
DETAILED DRUG EVALUATION, IN VITRO		40.00	24,306	
DET. DRUG EVAL., RELATED NEW MODEL DEVEL		30.00	18,230	
BIOLOGICAL STUDIES		* 30.00*	18,230	
BIOCHEMISTRY				
BRISTOL-MYERS COMPANY	N01CM77147 D.T.P.			333,000
ACQUISITION OF MATERIALS		* 85.00*	283,050	
NEW AGENT PROCUREMENT, FERMENT/ANTIBIOT		83.00	276,390	
DATA PROCESSING AND SUPPORT		2.00	6,660	
BASIC SCREEN		* 15.00*	49,950	

NAME		# & AREA	PERCENT OF EFFORT		DOLLAR LEVEL
PRIMARY SCREENING, IN VITRO			10.00	33,300	
PRIMARY SCREEN., RELATED NEW MODEL DEVEL			5.00	16,650	
BRITISH COLUMBIA, UNIVERSITY OF		N01CM23223 D.T.P.			109,985
ACQUISITION OF MATERIALS			* 80.00*	87,988	
NEW AGENT PROCUREMENT, SYNTHETICS					
PROCUREMENT OF PRECLINICAL MATERIAL			* 5.00*	5,499	
RADIOLABELED MATERIALS					
PHARMACOLOGY/TOXICOLOGY			* 15.00*	16,498	
METABOLITE ISOLATION					
CALIFORNIA, UNIVERSITY OF		N01CM53752 BCRC			133,618
PROGRAM MANAGEMENT			* 10.00*	13,362	
ADMINISTRATION					
PHASE II CLINICAL TRIALS			* 30.00*	40,085	
CNS TUMORS	RAD.				
PHASE III CLINICAL TRIALS			* 60.00*	80,171	
CNS TUMORS	RAD.		30.00	40,085	
CNS TUMORS	CHEM.		30.00	40,085	
CALIFORNIA, UNIVERSITY OF		N01CM02092 C.O.P.			400,000
CLINICAL TRIALS SUPPORTIVE RESEARCH			*100.00*	400,000	
NON-SPECIFIC	TRANSFUSION RES.				
CALIFORNIA, UNIVERSITY OF		N01CM67097 CTEP			155,216
PHASE IV CLINICAL TRIALS			*100.00*	155,216	
COLORECTAL	SURG.		20.00	31,043	
COLORECTAL	RAD.		15.00	23,282	
COLORECTAL	CHEM.		45.00	69,847	
COLORECTAL	IMM.		20.00	31,043	
CALIFORNIA, UNIVERSITY OF		N01CM77151 CTEP			109,003
PHASE II CLINICAL TRIALS			* 20.00*	21,801	
LUNG	RAD.		10.00	10,900	
LUNG	CHEM.		10.00	10,900	
PHASE III CLINICAL TRIALS			* 60.00*	65,402	
LUNG	RAD.		30.00	32,701	
LUNG	CHEM.		30.00	32,701	
PHASE IV CLINICAL TRIALS			* 20.00*	21,801	
LUNG	RAD.		10.00	10,900	
LUNG	CHEM.		10.00	10,900	
CALIFORNIA, UNIVERSITY OF		N01CM43791 D.T.P.			69,608
VERIFICATION SCREEN			* 50.00*	34,804	
DETAILED DRUG EVALUATION, IN VITRO			20.00	13,922	

NAME	# & AREA	PERCENT OF EFFORT		DOLLAR LEVEL
DET. DRUG EVAL., RELATED NEW MODEL DEVEL		30.00	20,882	
TREATMENT STUDIES		* 50.00*	34,804	
COMB. MODAL. THERAPY		50.00	34,804	
COMB. MODAL. THERAPY - RAD.				
CALIFORNIA, UNIVERSITY OF	N01HB62971 O.D.			151,313
CLINICAL TRIALS SUPPORTIVE RESEARCH		*100.00*	151,313	
OTHER TRANSFUSION RES.				
CALIFORNIA, UNIVERSITY OF	N01CM67055 BCRC			76,933
PROGRAM MANAGEMENT		* 10.00*	7,693	
ADMINISTRATION				
PHASE II CLINICAL TRIALS		* 30.00*	23,080	
CNS TUMORS RAD.				
PHASE III CLINICAL TRIALS		* 60.00*	46,160	
CNS TUMORS RAD.		30.00	23,080	
CNS TUMORS CHEM.		30.00	23,080	
CATHOLIC MEDICAL CTR OF BROOKLYN AND	N01CM67031 D.T.P.			514,600
BASIC SCREEN		* 40.00*	205,840	
PRIMARY SCREENING, IN VIVO		18.00	92,628	
ANIMALS		22.00	113,212	
VERIFICATION SCREEN		* 40.00*	205,840	
DETAILED DRUG EVALUATION, IN VIVO		18.00	92,628	
ANIMALS		22.00	113,212	
TREATMENT STUDIES		* 20.00*	102,920	
COMB. MODAL. THERAPY		20.00	102,920	
COMB. MODAL. THERAPY - SURG.		9.00	46,314	
COMB. MODAL. THERAPY - CHEM.		9.00	46,314	
COMB. MODAL. THERAPY - IMM.		2.00	10,292	
CHARLES RIVER BREEDING LABS.	N01CM53812 A.P.			1,550,000
ACQUISITION OF MATERIALS		* 20.00*	310,000	
ANIMALS				
BASIC SCREEN		* 55.00*	852,500	
ANIMALS				
VERIFICATION SCREEN		* 25.00*	387,500	
ANIMALS				
CHARLES RIVER BREEDING LABS.	N01CM50598 A.P.			251,160
ACQUISITION OF MATERIALS		* 20.00*	50,232	
ANIMALS				
BASIC SCREEN		* 55.00*	138,138	
ANIMALS				
VERIFICATION SCREEN		* 25.00*	62,790	
ANIMALS				

NAME		# & AREA	PERCENT OF EFFORT		DOLLAR LEVEL
CHARLES RIVER BREEDING LABS.		N01CM53848 A.P.			108,000
ACQUISITION OF MATERIALS			* 20.00*	21,600	
ANIMALS					
BASIC SCREEN			* 55.00*	59,400	
ANIMALS					
VERIFICATION SCREEN			* 25.00*	27,000	
ANIMALS					
CHEMICAL ABSTRACTS SERVICE		N01CM43722 D.T.P.			449,100
ACQUISITION OF MATERIALS			*100.00*	449,100	
DATA PROCESSING AND SUPPORT					
CHESTER BEATTY RESEARCH INSTITUTE		N01CM43736 D.T.P.			32,000
ACQUISITION OF MATERIALS			* 50.00*	16,000	
NEW AGENT PROCUREMENT, SYNTHETICS			40.00	12,800	
DATA PROCESSING AND SUPPORT			10.00	3,200	
VERIFICATION SCREEN			* 50.00*	16,000	
DETAILED DRUG EVALUATION, IN VITRO			5.00	1,600	
DETAILED DRUG EVALUATION, IN VIVO			20.00	6,400	
DET. DRUG EVAL., RELATED NEW MODEL DEVEL			25.00	8,000	
CHICAGO, UNIVERSITY OF		N01CM67095 CTEP			191,315
PHASE IV CLINICAL TRIALS			*100.00*	191,315	
COLORECTAL	SURG.		20.00	38,263	
COLORECTAL	RAD.		15.00	28,697	
COLORECTAL	CHEM.		45.00	86,092	
COLORECTAL	IMM.		20.00	38,263	
CHILDREN'S HOSPITAL MEDICAL CENTER		N01CP65826 CTEP			34,372
CLINICAL TRIALS SUPPORTIVE RESEARCH			*100.00*	34,372	
HEAD AND NECK	NUTRITION		20.00	6,874	
LUNG	NUTRITION		20.00	6,874	
OVARIAN	NUTRITION		20.00	6,874	
PANCREATIC	NUTRITION		20.00	6,874	
STOMACH	NUTRITION		20.00	6,874	
CHILDREN'S HOSPITAL OF LOS ANGELES		N01CM53831 C.O.P.			24,402
CLINICAL TRIALS SUPPORTIVE RESEARCH			*100.00*	24,402	
NEUROBLASTOMA	PROTECTED ENVIRON.		50.00	12,201	
RHABDOMYOSARCOMA	PROTECTED ENVIRON.		50.00	12,201	
CHILDREN'S HOSPITAL OF LOS ANGELES		N01CM43803 CTEP			98,173
PHASE I CLINICAL TRIALS			*100.00*	98,173	

NAME		# & AREA	PERCENT OF EFFORT	DOLLAR LEVEL	
LEUK.-ACUTE GRAN.	CHEM.		30.00	29,452	
LEUK.-ACUTE LYMPH.	CHEM.		30.00	29,452	
NEUROBLASTOMA	CHEM.		10.00	9,817	
RHABDOMYOSARCOMA	CHEM.		20.00	19,635	
WILMS' TUMOR	CHEM.		10.00	9,817	
CHILDREN'S HOSPITAL OF LOS ANGELES		N01CP65828 CTEP			62,749
CLINICAL TRIALS SUPPORTIVE RESEARCH			*100.00*	62,749	
HEAD AND NECK	NUTRITION		20.00	12,550	
LUNG	NUTRITION		20.00	12,550	
OVARIAN	NUTRITION		20.00	12,550	
PANCREATIC	NUTRITION		20.00	12,550	
STOMACH	NUTRITION		20.00	12,550	
CLINICA NEUROCHIRURGICA DELL		N01CM67056 BCRC			22,266
PROGRAM MANAGEMENT ADMINISTRATION			* 10.00*	2,227	
PHASE II CLINICAL TRIALS			* 30.00*	6,680	
CNS TUMORS	RAD.				
PHASE III CLINICAL TRIALS			* 60.00*	13,360	
CNS TUMORS	RAD.		30.00	6,680	
CNS TUMORS	CHEM.		30.00	6,680	
COLLABORATIVE RESEARCH, INC.		N01CM77071 D.T.P.			94,271
ACQUISITION OF MATERIALS NEW AGENT PROCUREMENT, SYNTHETICS			* 90.00*	84,844	
PROCUREMENT OF PRECLINICAL MATERIAL SYNTHETICS			* 10.00*	9,427	
COLORADO STATE UNIVERSITY		N01CM12192 D.T.P.			10,772
PHARMACOLOGY/TOXICOLOGY SPECIAL TOXICITY STUDIES			*100.00*	10,772	
COLORADO, UNIVERSITY OF, MEDICAL CENTER		N01CM12186 D.T.P.			125,000
BASIC SCREEN PRIMARY SCREENING, IN VITRO			* 30.00*	37,500	
BIOLOGICAL STUDIES MOLECULAR BIOLOGY			* 70.00*	87,500	
CONTROL DATA CORPORATION		N01CM67107 BCRC			50,000
PROGRAM MANAGEMENT ADMINISTRATION			* 10.00*	5,000	
OTHER CLINICAL TRIALS RESEARCH			* 90.00*	45,000	
CNS TUMORS	DATA PROC. & SUPP.				

NAME	# & AREA	PERCENT OF EFFORT		DOLLAR LEVEL
CORDOVA CHEMICAL COMPANY	N01CM57031 D.T.P.			100,600
PROCUREMENT OF PRECLINICAL MATERIAL SYNTHETICS		* 25.00*	25,150	
PROD. AND FORM. FOR CLINICAL TRIALS PRODUCTION, SYNTHETICS		* 75.00*	75,450	
DOT SYSTEMS, INC.	N01CM53774 O.D.			79,617
PROGRAM MANAGEMENT COMMUNICATION AND EDUCATION		*100.00*	79,617	
DOW CHEMICAL COMPANY	N01CM53768 D.T.P.			90,000
PROCUREMENT OF PRECLINICAL MATERIAL SYNTHETICS		* 25.00*	22,500	
PROD. AND FORM. FOR CLINICAL TRIALS PRODUCTION, SYNTHETICS		* 75.00*	67,500	
DOW CHEMICAL COMPANY	N01CM23712 D.T.P.			172,718
PHARMACOLOGY/TOXICOLOGY SPECIAL TOXICITY STUDIES		*100.00*	172,718	
DUKE UNIVERSITY	N01CM67010 BCRC			98,529
PROGRAM MANAGEMENT ADMINISTRATION		* 10.00*	9,853	
PHASE II CLINICAL TRIALS CNS TUMORS RAD.		* 30.00*	29,559	
PHASE III CLINICAL TRIALS		* 60.00*	59,117	
CNS TUMORS RAD.		30.00	29,559	
CNS TUMORS CHEM.		30.00	29,559	
ELKINS-SINN, INC.	N01CM67103 D.T.P.			147,233
FORMULATION DEVEL. OF EXP. FORMULATIONS		* 20.00*	29,447	
PROD. AND FORM. FOR CLINICAL TRIALS		* 80.00*	117,786	
FORMULATION		70.00	103,063	
ANALYTICAL AND QUALITY CONTROL		10.00	14,723	
ENERGY RES & DEV ADM	Y01CM10060 D.T.P.			35,410
CLINICAL TRIALS SUPPORTIVE RESEARCH NON-SPECIFIC MARKERS		*100.00*	35,410	
ENERGY RES & DEV ADM	Y01CM10056 D.T.P.			27,500
VERIFICATION SCREEN		*100.00*	27,500	

NAME	# & AREA	PERCENT OF EFFORT		DOLLAR LEVEL
DETAILED DRUG EVALUATION, IN VITRO		70.00	19,250	
DET. DRUG EVAL., RELATED NEW MODEL DEVEL		30.00	8,250	
ENERGY RES & DEV ADM	Y01CM40102 O.D.			105,000
BIOLOGICAL STUDIES CELL KINETICS		* 10.00*	10,500	
CLINICAL TRIALS SUPPORTIVE RESEARCH NON-SPECIFIC CELL KINETICS		* 90.00*	94,500	
ENERGY RES & DEV ADM	Y01CM40101 O.D.			12,800
CLINICAL TRIALS SUPPORTIVE RESEARCH NON-SPECIFIC BLOOD PRODUCTS		*100.00*	12,800	
FARMITALIA	N01CM57036 D.T.P.			189,590
ACQUISITION OF MATERIALS NEW AGENT PROCUREMENT, SYNTHETICS		* 90.00*	170,631	
PROCUREMENT OF PRECLINICAL MATERIAL SYNTHETICS		* 10.00*	18,959	
FARMITALIA	N01CM57014 D.T.P.			193,163
ACQUISITION OF MATERIALS NEW AGENT PROCUREMENT, SYNTHETICS		* 90.00*	173,847	
PROCUREMENT OF PRECLINICAL MATERIAL SYNTHETICS		* 10.00*	19,316	
FLOW LABORATORIES, INC.	N01CM50571 A.P.			219,960
ACQUISITION OF MATERIALS ANIMALS		* 20.00*	43,992	
BASIC SCREEN ANIMALS		* 55.00*	120,978	
VERIFICATION SCREEN ANIMALS		* 25.00*	54,990	
FLOW LABORATORIES, INC.	N01CM57033 D.T.P.			122,180
PROD. AND FORM. FOR CLINICAL TRIALS STOR.&DIST. OF DRUGS		*100.00*	122,180	
FRANKLIN INSTITUTE RESEARCH LABS.	N01CM77092 O.D.			401,310
PROGRAM MANAGEMENT COMMUNICATION AND EDUCATION		*100.00*	401,310	
FRED HUTCHINSON CANCER RESEARCH	N01CM77148 CTEP			115,815
PHASE II CLINICAL TRIALS		* 20.00*	23,163	

NAME		# & AREA	PERCENT OF EFFORT		DOLLAR LEVEL
LUNG	RAD.		10.00	11,582	
LUNG	CHEM.		10.00	11,582	
PHASE III CLINICAL TRIALS			* 60.00*	69,489	
LUNG	RAD.		30.00	34,745	
LUNG	CHEM.		30.00	34,745	
PHASE IV CLINICAL TRIALS			* 20.00*	23,163	
LUNG	RAD.		10.00	11,582	
LUNG	CHEM.		10.00	11,582	
FRONTIER SCIENCE & TECHNOLOGY RES.		N01CM77136 CTEP			170,991
CLINICAL TRIALS SUPPORTIVE RESEARCH			*100.00*	170,991	
LUNG	DATA PROC. & SUPP.				
GEORGETOWN UNIVERSITY		N01CM33745 CTEP			175,314
CLINICAL TRIALS SUPPORTIVE RESEARCH			*100.00*	175,314	
NON-SPECIFIC	INFECTIOUS DISEASE				
GEORGETOWN UNIVERSITY		N01CM67094 CTEP			88,609
PHASE IV CLINICAL TRIALS			*100.00*	88,609	
COLORECTAL	SURG.		20.00	17,722	
COLORECTAL	RAD.		15.00	13,291	
COLORECTAL	CHEM.		45.00	39,874	
COLORECTAL	IMM.		20.00	17,722	
GEORGETOWN UNIVERSITY		N01CM67110 CTEP			218,019
PHARMACOLOGY/TOXICOLOGY			* 10.00*	21,802	
PHARMACOLOGY					
PHASE I CLINICAL TRIALS			* 15.00*	32,703	
COLORECTAL	CHEM.		5.00	10,901	
PANCREATIC	CHEM.		5.00	10,901	
STOMACH	CHEM.		5.00	10,901	
PHASE II CLINICAL TRIALS			* 15.00*	32,703	
COLORECTAL	CHEM.		5.00	10,901	
PANCREATIC	CHEM.		5.00	10,901	
STOMACH	CHEM.		5.00	10,901	
PHASE III CLINICAL TRIALS			* 30.00*	65,406	
COLORECTAL	SURG.		2.00	4,360	
COLORECTAL	RAD.		2.00	4,360	
COLORECTAL	CHEM.		2.00	4,360	
COLORECTAL	IMM.		2.00	4,360	
COLORECTAL	SUPPORTIVE CARE		2.00	4,360	
PANCREATIC	SURG.		2.00	4,360	
PANCREATIC	RAD.		2.00	4,360	
PANCREATIC	CHEM.		2.00	4,360	
PANCREATIC	IMM.		2.00	4,360	
PANCREATIC	SUPPORTIVE CARE		2.00	4,360	
STOMACH	SURG.		2.00	4,360	

NAME	# & AREA	PERCENT OF EFFORT		DOLLAR LEVEL
STOMACH RAD.		2.00	4,360	
STOMACH CHEM.		2.00	4,360	
STOMACH IMM.		2.00	4,360	
STOMACH SUPPORTIVE CARE		2.00	4,360	
CLINICAL TRIALS SUPPORTIVE RESEARCH		* 30.00*	65,406	
COLORECTAL NUTRITION		10.00	21,802	
PANCREATIC NUTRITION		10.00	21,802	
STOMACH NUTRITION		10.00	21,802	
GEORGETOWN UNIVERSITY	N01CM43732 D.T.P.			59,985
ACQUISITION OF MATERIALS		*100.00*	59,985	
NEW AGENT PROCUREMENT, SYNTHETICS		25.00	14,996	
NEW AGENT PROCUREMENT, FERMENT/ANTIBIOT		75.00	44,989	
GRACE, W.R.	N01CM67074 D.T.P.			221,182
ACQUISITION OF MATERIALS		*100.00*	221,182	
NEW AGENT PROCUREMENT, MARINE/ARTHROPODS				
HARLAN INDUSTRIES	N01CM50591 A.P.			226,200
ACQUISITION OF MATERIALS		* 20.00*	45,240	
ANIMALS				
BASIC SCREEN		* 55.00*	124,410	
ANIMALS				
VERIFICATION SCREEN		* 25.00*	56,550	
ANIMALS				
HARLAN INDUSTRIES	N01CM67079 A.P.			136,310
BASIC SCREEN		*100.00*	136,310	
ANIMALS				
HARVARD COLLEGE	N01CM53825 D.T.P.			51,853
VERIFICATION SCREEN		* 30.00*	15,556	
DET. DRUG EVAL., RELATED NEW MODEL DEVEL				
BIOLOGICAL STUDIES		* 70.00*	36,297	
MOLECULAR BIOLOGY				
HAWAII, UNIVERSITY OF	N01CM33747 D.T.P.			82,246
ACQUISITION OF MATERIALS		*100.00*	82,246	
NEW AGENT PROCUREMENT, PLANT PRODUCTS				
HAZLETON LABORATORIES, INC.	N01CM60125 A.P.			37,500
PHARMACOLOGY/TOXICOLOGY		*100.00*	37,500	
ANIMALS				

NAME		# & AREA	PERCENT OF EFFORT		DOLLAR LEVEL
HAZLETON LABORATORIES, INC.		N01CM67080 BCRC			425,574
PROGRAM MANAGEMENT			* 3.00*	12,767	
ADMINISTRATION			1.00	4,256	
DATA PROCESSING AND SUPPORT			2.00	8,511	
CLINICAL TRIALS SUPPORTIVE RESEARCH			* 97.00*	412,807	
LEUK.-ACUTE GRAN.	MICROBIOLOGY		33.00	140,439	
LEUK.-ACUTE LYMPH.	MICROBIOLOGY		5.00	21,279	
LEUK.-CHRON. GRAN.	MICROBIOLOGY		10.00	42,557	
LEUK.-CHRON LYMPH.	MICROBIOLOGY		5.00	21,279	
LYMPHOMA-HISTIOCYT	MICROBIOLOGY		2.00	8,511	
LYMPHOMA-HODGKIN'S	MICROBIOLOGY		10.00	42,557	
LYMPHOMA-LYMPHOCYT	MICROBIOLOGY		3.00	12,767	
LYMPHOMA-MYCO.FUNG	MICROBIOLOGY		4.00	17,023	
MYELOMA	MICROBIOLOGY		4.00	17,023	
CNS TUMORS	MICROBIOLOGY		5.00	21,279	
COLORECTAL	MICROBIOLOGY		6.00	25,534	
KIDNEY	MICROBIOLOGY		5.00	21,279	
LUNG	MICROBIOLOGY		3.00	12,767	
MELANOMA	MICROBIOLOGY		2.00	8,511	
HAZLETON LABORATORIES, INC.		N01CM77126 C.O.P.			42,896
BIOLOGICAL STUDIES			* 70.00*	30,027	
BIOCHEMISTRY			20.00	8,579	
CELL KINETICS			20.00	8,579	
MARKERS			10.00	4,290	
NUTRITION			20.00	8,579	
TREATMENT STUDIES			* 30.00*	12,869	
SURGERY			10.00	4,290	
RADIOTHERAPY			10.00	4,290	
CHEMOTHERAPY			10.00	4,290	
HAZLETON LABORATORIES, INC.		N01CM43702 C.O.P.			135,820
BIOLOGICAL STUDIES			* 50.00*	67,910	
TRANSFUSION RESEARCH			50.00	67,910	
TREATMENT STUDIES			* 50.00*	67,910	
MARROW TRANSPLANT.					
HAZLETON LABORATORIES, INC.		N01CM33708 D.T.P.			400,005
ACQUISITION OF MATERIALS			* 20.00*	80,001	
ANIMALS					
VERIFICATION SCREEN			* 10.00*	40,001	
ANIMALS					
PHARMACOLOGY/TOXICOLOGY			* 40.00*	160,002	
PHARMACOLOGY			10.00	40,001	
SPECIAL TOXICITY STUDIES			30.00	120,002	
BIOLOGICAL STUDIES			* 30.00*	120,002	

NAME		# & AREA	PERCENT OF EFFORT	DOLLAR LEVEL	
MARKERS					
HAZLETON RESEARCH ANIMALS, INC.		N01CM77144 C.O.P.			24,822
TREATMENT STUDIES			*100.00*	24,822	
CHEMOTHERAPY					
HEALTH RESEARCH, INC.		N01CM53834 CTEP			52,852
PHASE II CLINICAL TRIALS			*100.00*	52,852	
BREAST	CHEM.				
HEALTH RESEARCH, INC.		N01CM43794 CTEP			34,817
PHASE II CLINICAL TRIALS			* 30.00*	10,445	
PANCREATIC	CHEM.				
PHASE IV CLINICAL TRIALS			* 50.00*	17,409	
PANCREATIC	SURG.		5.00	1,741	
PANCREATIC	RAD.		20.00	6,963	
PANCREATIC	CHEM.		25.00	8,704	
OTHER CLINICAL TRIALS RESEARCH			* 20.00*	6,963	
PANCREATIC	DATA PROC. & SUPP.				
HEALTH RESEARCH, INC.		N01CM77101 A.P.			85,972
ACQUISITION OF MATERIALS			* 20.00*	17,194	
ANIMALS					
BASIC SCREEN			* 55.00*	47,285	
ANIMALS					
VERIFICATION SCREEN			* 25.00*	21,493	
ANIMALS					
HEALTH RESEARCH, INC.		N01CM43782 CTEP			96,141
PHASE IV CLINICAL TRIALS			* 90.00*	86,527	
STOMACH	SURG.		25.00	24,035	
STOMACH	RAD.		25.00	24,035	
STOMACH	CHEM.		40.00	38,456	
CLINICAL TRIALS SUPPORTIVE RESEARCH			* 10.00*	9,614	
STOMACH	MARKERS				
HEALTH RESEARCH, INC.		N01CM57034 CTEP			137,023
PHASE IV CLINICAL TRIALS			*100.00*	137,023	
COLORECTAL	SURG.		20.00	27,405	
COLORECTAL	RAD.		15.00	20,553	
COLORECTAL	CHEM.		45.00	61,660	
COLORECTAL	IMM.		20.00	27,405	

NAME		# & AREA	PERCENT OF EFFORT		DOLLAR LEVEL
HEALTH RESEARCH, INC.		N01CM67117 CTEP			124,175
PHASE III CLINICAL TRIALS			* 50.00*	62,088	
OVARIAN	SURG.		15.00	18,626	
OVARIAN	RAD.		15.00	18,626	
OVARIAN	CHEM.		20.00	24,835	
PHASE IV CLINICAL TRIALS			* 50.00*	62,088	
OVARIAN	SURG.		15.00	18,626	
OVARIAN	RAD.		15.00	18,626	
OVARIAN	CHEM.		20.00	24,835	
HEALTH RESEARCH, INC.		N01CM33726 D.T.P.			45,235
BASIC SCREEN			* 80.00*	36,188	
PRIMARY SCREENING, IN VITRO			60.00	27,141	
PRIMARY SCREENING, IN VIVO			20.00	9,047	
PHARMACOLOGY/TOXICOLOGY			* 20.00*	9,047	
PHARMACOLOGY					
HEALTH RESEARCH, INC.		N01CM43785 D.T.P.			58,000
VERIFICATION SCREEN			*100.00*	58,000	
DET. DRUG EVAL., RELATED NEW MODEL DEVEL					
HEALTH RESEARCH, INC.		N01CM57039 D.T.P.			104,515
BIOLOGICAL STUDIES			*100.00*	104,515	
IMMUNOBIOLOGY			40.00	41,806	
IMMUNOSUPPRESSION			60.00	62,709	
HEBREW UNIVERSITY		N01CM12127 C.O.P.			50,000
PHASE III CLINICAL TRIALS			*100.00*	50,000	
LEUK.-ACUTE GRAN.	CHEM.		20.00	10,000	
LEUK.-ACUTE GRAN.	IMM.		20.00	10,000	
LYMPHOMA-HISTIOCYT	CHEM.		20.00	10,000	
LYMPHOMA-HISTIOCYT	IMM.		20.00	10,000	
LYMPHOMA-LYMPHOCYT	CHEM.		10.00	5,000	
LYMPHOMA-LYMPHOCYT	IMM.		10.00	5,000	
HEM RESEARCH		N01CM67066 D.T.P.			106,699
PROCUREMENT OF PRECLINICAL MATERIAL			* 50.00*	53,350	
VIRUS & CELLS					
BIOLOGICAL STUDIES			* 50.00*	53,350	
MARKERS			10.00	10,670	
MOLECULAR BIOLOGY			40.00	42,680	

NAME	# & AREA	PERCENT OF EFFORT		DOLLAR LEVEL
IIT RESEARCH INSTITUTE	N01CM53829			129,063
	A.P.			
ACQUISITION OF MATERIALS		* 20.00*	25,813	
ANIMALS				
BASIC SCREEN		* 55.00*	70,985	
ANIMALS				
VERIFICATION SCREEN		* 25.00*	32,266	
ANIMALS				
IIT RESEARCH INSTITUTE	N01CM43755			284,870
	D.T.P.			
ACQUISITION OF MATERIALS		* 20.00*	56,974	
BIOASSAY OF NATURAL PRODUCTS				
BASIC SCREEN		* 65.00*	185,166	
PRIMARY SCREENING, IN VITRO		5.00	14,244	
PRIMARY SCREENING, IN VIVO		55.00	156,679	
DATA PROCESSING AND SUPPORT		5.00	14,244	
VERIFICATION SCREEN		* 15.00*	42,731	
DETAILED DRUG EVALUATION, IN VIVO				
IIT RESEARCH INSTITUTE	N01CM63832			82,150
	D.T.P.			
ACQUISITION OF MATERIALS		* 5.00*	4,108	
BIOASSAY OF NATURAL PRODUCTS				
BASIC SCREEN		* 95.00*	78,043	
PRIMARY SCREENING, IN VITRO		90.00	73,935	
PRIMARY SCREEN., RELATED NEW MODEL DEVEL		5.00	4,108	
IIT RESEARCH INSTITUTE	N01CM57004			865,053
	D.T.P.			
ACQUISITION OF MATERIALS		* 15.00*	129,758	
BIOASSAY OF NATURAL PRODUCTS				
BASIC SCREEN		* 71.00*	614,188	
PRIMARY SCREENING, IN VIVO		64.00	553,634	
PRIMARY SCREEN., RELATED NEW MODEL DEVEL		7.00	60,554	
VERIFICATION SCREEN		* 11.00*	95,156	
DETAILED DRUG EVALUATION, IN VIVO				
TREATMENT STUDIES		* 3.00*	25,952	
CHEMOTHERAPY				
ILLINOIS, UNIVERSITY OF	N01CM67090			113,099
	D.T.P.			
ACQUISITION OF MATERIALS		* 85.00*	96,134	
NEW AGENT PROCUREMENT, PLANT PRODUCTS		65.00	73,514	
NEW AGENT PROCUREMENT, FERMENT/ANTIBIOT		15.00	16,965	
BIOASSAY OF NATURAL PRODUCTS		5.00	5,655	
PROCUREMENT OF PRECLINICAL MATERIAL		* 15.00*	16,965	
PLANT PRODUCTS		10.00	11,310	
FERMENTATION/ANTIBIOTICS		5.00	5,655	

NAME	# & AREA	PERCENT OF EFFORT		DOLLAR LEVEL
INDIANA UNIVERSITY FOUNDATION	N01CM67057 BCRC			33,696
PROGRAM MANAGEMENT		* 10.00*	3,370	
ADMINISTRATION				
PHASE II CLINICAL TRIALS		* 30.00*	10,109	
CNS TUMORS RAD.				
PHASE III CLINICAL TRIALS		* 60.00*	20,218	
CNS TUMORS RAD.		30.00	10,109	
CNS TUMORS CHEM.		30.00	10,109	
INFORMATION PLANNING ASSOC.	N01CM77104 CTEP			90,195
PROGRAM MANAGEMENT		*100.00*	90,195	
DATA PROCESSING AND SUPPORT				
INSTITUT JULES BORDET	N01CM53840 D.T.P.			93,900
ACQUISITION OF MATERIALS		* 30.00*	28,170	
NEW AGENT PROCUREMENT, SYNTHETICS		25.00	23,475	
NEW AGENT PROCUREMENT, FERMENT/ANTIBIOT		5.00	4,695	
BIOLOGICAL STUDIES		* 20.00*	18,780	
DRUG EVAL./INVEST.				
PROGRAM MANAGEMENT		* 50.00*	46,950	
COMMUNICATION AND EDUCATION		30.00	28,170	
RESOURCE DEVELOPMENT		5.00	4,695	
DATA PROCESSING AND SUPPORT		15.00	14,085	
INSTITUT JULES BORDET	N01CM57040 D.T.P.			288,124
BASIC SCREEN		* 80.00*	230,499	
PRIMARY SCREENING, IN VIVO				
VERIFICATION SCREEN		* 20.00*	57,625	
DETAILED DRUG EVALUATION, IN VIVO				
INSTITUTO NAZIONALE LA CURA DEI TOMORI	N01CM67115 C.O.P.			21,715
CLINICAL TRIALS SUPPORTIVE RESEARCH		*100.00*	21,715	
LYMPHOMA-HISTIOCYT PATHOLOGIC CLASSIF		50.00	10,858	
LYMPHOMA-LYMPHOCYT PATHOLOGIC CLASSIF		50.00	10,858	
IOWA, UNIVERSITY OF	N01CM43743 D.T.P.			193,502
FORMULATION		* 20.00*	38,700	
DEVEL. OF EXP. FORMULATIONS				
PROD. AND FORM. FOR CLINICAL TRIALS		* 80.00*	154,802	
FORMULATION		70.00	135,451	
ANALYTICAL AND QUALITY CONTROL		10.00	19,350	

NAME		# & AREA	PERCENT OF EFFORT		DOLLAR LEVEL
IOWA, UNIVERSTIY OF		N01HB62973 O.D.			135,964
CLINICAL TRIALS SUPPORTIVE RESEARCH			*100.00*	135,964	
OTHER	TRANSFUSION RES.				
IST. NAZION PER LO STUD E LA CURA DEI		N01CM43726 CTEP			95,375
PHASE II CLINICAL TRIALS			* 40.00*	38,150	
MELANOMA	CHEM.				
PHASE IV CLINICAL TRIALS			* 60.00*	57,225	
MELANOMA	SURG.		20.00	19,075	
MELANOMA	CHEM.		20.00	19,075	
MELANOMA	IMM.		20.00	19,075	
IST. NAZION PER LO STUD E LA CURA DEI		N01CM33714 CTEP			145,069
PHASE II CLINICAL TRIALS			* 15.00*	21,760	
COLORECTAL	CHEM.		10.00	14,507	
STOMACH	CHEM.		5.00	7,253	
PHASE III CLINICAL TRIALS			* 35.00*	50,774	
BREAST	CHEM.				
PHASE IV CLINICAL TRIALS			* 50.00*	72,535	
BREAST	SURG.		16.00	23,211	
BREAST	RAD.		6.00	8,704	
BREAST	CHEM.		16.00	23,211	
CNS TUMORS	SURG..		4.00	5,803	
CNS TUMORS	RAD.		4.00	5,803	
CNS TUMORS	CHEM.		4.00	5,803	
JACKSON LABORATORY		N01CM60176 A.P.			153,660
ACQUISITION OF MATERIALS			* 20.00*	30,732	
ANIMALS					
BASIC SCREEN			* 55.00*	84,513	
ANIMALS					
VERIFICATION SCREEN			* 25.00*	38,415	
ANIMALS					
JAPANESE FOUNDATION FOR CANCER RESEARCH		N01CM22054 D.T.P.			70,500
ACQUISITION OF MATERIALS			* 10.00*	7,050	
NEW AGENT PROCUREMENT, SYNTHETICS			5.00	3,525	
NEW AGENT PROCUREMENT, FERMENT/ANTIBIOT			5.00	3,525	
BASIC SCREEN			* 40.00*	28,200	
PRIMARY SCREENING, IN VITRO			2.00	1,410	
PRIMARY SCREENING, IN VIVO			18.00	12,690	
DRUG EVALUATION			20.00	14,100	
PROGRAM MANAGEMENT			* 20.00*	14,100	
COMMUNICATION AND EDUCATION			10.00	7,050	
RESOURCE DEVELOPMENT			10.00	7,050	

NAME		# & AREA	PERCENT OF EFFORT		DOLLAR LEVEL
PHASE I CLINICAL TRIALS			* 15.00*	10,575	
LEUK.-ACUTE GRAN.	CHEM.		5.00	3,525	
LEUK.-ACUTE LYMPH.	CHEM.		5.00	3,525	
NON-SPECIFIC	CHEM.		5.00	3,525	
PHASE II CLINICAL TRIALS			* 15.00*	10,575	
LEUK.-ACUTE GRAN.	CHEM.		5.00	3,525	
LEUK.-ACUTE LYMPH.	CHEM.		5.00	3,525	
NON-SPECIFIC	CHEM.		5.00	3,525	
JOHNS HOPKINS UNIVERSITY		N01CM43718 D.T.P.			43,050
BIOLOGICAL STUDIES			*100.00*	43,050	
MARKERS					
KANSAS, UNIVERSITY OF		N01CM43802 CTEP			84,652
PHASE I CLINICAL TRIALS			*100.00*	84,652	
NON-SPECIFIC	CHEM.				
KANSAS, UNIVERSITY OF		N01CM12184 D.T.P.			56,500
CLINICAL TRIALS SUPPORTIVE RESEARCH			*100.00*	56,500	
NON-SPECIFIC	SPEC. PHARM./TOX.				
KANSAS, UNIVERSITY OF		N01CM23217 D.T.P.			20,840
FORMULATION			*100.00*	20,840	
DEVEL. OF EXP. FORMULATIONS					
KENTUCKY, UNIVERSITY OF		N01CM67058 BCRC			45,360
PROGRAM MANAGEMENT			* 10.00*	4,536	
ADMINISTRATION					
PHASE II CLINICAL TRIALS			* 30.00*	13,608	
CNS TUMORS	RAD.				
PHASE III CLINICAL TRIALS			* 60.00*	27,216	
CNS TUMORS	RAD.		30.00	13,608	
CNS TUMORS	CHEM.		30.00	13,608	
KENTUCKY, UNIVERSITY OF		N01CM67038 D.T.P.			159,075
CLINICAL TRIALS SUPPORTIVE RESEARCH			*100.00*	159,075	
LEUK.-ACUTE LYMPH.	MARKERS				
LABORATORY RESEARCH ENTERPRISES, INC.		N01CM60124 A.P.			36,875
PHARMACOLOGY/TOXICOLOGY			*100.00*	36,875	
ANIMALS					

NAME		# & AREA	PERCENT OF EFFORT		DOLLAR LEVEL
LABORATORY SUPPLY CO.		N01CM53775			142,796
		A.P.			
ACQUISITION OF MATERIALS			* 20.00*	28,559	
ANIMALS					
BASIC SCREEN			* 55.00*	78,538	
ANIMALS					
VERIFICATION SCREEN			* 25.00*	35,699	
ANIMALS					
LABORATORY SUPPLY CO.		N01CM50577			230,880
		A.P.			
ACQUISITION OF MATERIALS			* 20.00*	46,176	
ANIMALS					
BASIC SCREEN			* 55.00*	126,984	
ANIMALS					
VERIFICATION SCREEN			* 25.00*	57,720	
ANIMALS					
LEEDS, UNIVERSITY OF		N01CM43727			32,900
		CTEP			
PHASE II CLINICAL TRIALS			* 50.00*	16,450	
COLORECTAL	CHEM.		38.00	12,502	
STOMACH	CHEM.		12.00	3,948	
CLINICAL TRIALS SUPPORTIVE RESEARCH			* 50.00*	16,450	
COLORECTAL	MARKERS		38.00	12,502	
STOMACH	MARKERS		12.00	3,948	
LEO GOODWIN INST. FOR CANCER RESEARCH		N01CM53814			480,000
		A.P.			
ACQUISITION OF MATERIALS			* 20.00*	96,000	
ANIMALS					
BASIC SCREEN			* 55.00*	264,000	
ANIMALS					
VERIFICATION SCREEN			* 25.00*	120,000	
ANIMALS					
LITTON BIONETICS, INC.		N01CM53773			76,305
		A.P.			
ACQUISITION OF MATERIALS			* 20.00*	15,261	
ANIMALS					
BASIC SCREEN			* 55.00*	41,968	
ANIMALS					
VERIFICATION SCREEN			* 25.00*	19,076	
ANIMALS					
LITTON BIONETICS, INC.		N01CM67067			311,778
		C.O.P.			
PROD. AND FORM. FOR CLINICAL TRIALS			* 10.00*	31,178	
DATA PROCESSING AND SUPPORT					
BIOLOGICAL STUDIES			* 90.00*	280,600	

NAME	# & AREA	PERCENT OF EFFORT		DOLLAR LEVEL
IMMUNOLOGY				
LITTON BIONETICS, INC.	N01CM77134 D.T.P.			731,124
PROCUREMENT OF PRECLINICAL MATERIAL		* 50.00*	365,562	
ANTIBODIES/ANTIGENS				
BIOLOGICAL STUDIES		* 50.00*	365,562	
MOLECULAR BIOLOGY				
LITTON BIONETICS, INC.	N01CM22062 D.T.P.			257,926
BIOLOGICAL STUDIES		*100.00*	257,926	
BLOOD PRODUCTS		40.00	103,170	
TISSUE CULTURE CELLS		60.00	154,756	
LITTON BIONETICS, INC.	N01CM12340 D.T.P.			849,986
PROCUREMENT OF PRECLINICAL MATERIAL		* 50.00*	424,993	
ANTIBODIES/ANTIGENS				
BIOLOGICAL STUDIES		* 50.00*	424,993	
MOLECULAR BIOLOGY				
LITTON BIONETICS, INC.	N01CO25423 O.D.			5,000,000
ACQUISITION OF MATERIALS		* 48.00*	2,400,000	
NEW AGENT PROCUREMENT, FERMENT/ANTIBIOT				
BASIC SCREEN		* 12.00*	600,000	
PRIMARY SCREENING, IN VITRO		3.00	150,000	
ANIMALS		9.00	450,000	
VERIFICATION SCREEN		* 30.00*	1,500,000	
ANIMALS				
PROCUREMENT OF PRECLINICAL MATERIAL		* 10.00*	500,000	
FERMENTATION/ANTIBIOTICS				
MADRID, UNIVERSITY OF	N01CM53792 D.T.P.			18,528
VERIFICATION SCREEN		*100.00*	18,528	
DETAILED DRUG EVALUATION, IN VITRO		70.00	12,970	
DETAILED DRUG EVALUATION, IN VIVO		10.00	1,853	
DET. DRUG EVAL., RELATED NEW MODEL DEVEL		20.00	3,706	
MAKERERE UNIVERSITY COLLEGE COUNCIL	N01CM71343 C.O.P.			67,252
PHASE III CLINICAL TRIALS		*100.00*	67,252	
LYMPHOMA-BURKITT'S CHEM.		60.00	40,351	
LYMPHOMA-HODGKIN'S CHEM.		20.00	13,450	
HEPATOBILIARY CHEM.		20.00	13,450	
MARIO NEGRI INST OF PHARMACOLOGICAL	N01CM33720 D.T.P.			193,110
ACQUISITION OF MATERIALS		* 15.00*	28,967	

NAME		# & AREA	PERCENT OF EFFORT		DOLLAR LEVEL
NEW AGENT PROCUREMENT, SYNTHETICS					
BASIC SCREEN			* 70.00*	135,177	
PRIMARY SCREENING, IN VIVO					
VERIFICATION SCREEN			* 15.00*	28,967	
DETAILED DRUG EVALUATION, IN VIVO			10.00	19,311	
DET. DRUG EVAL., RELATED NEW MODEL DEVEL			5.00	9,656	
MARIO NEGRI INST OF PHARMACOLOGICAL		N01CM67064 D.T.P.			75,580
PHARMACOLOGY/TOXICOLOGY			*100.00*	75,580	
PHARMACOLOGY					
MARIO NEGRI INST OF PHARMACOLOGICAL		N01CM53826 D.T.P.			96,809
TREATMENT STUDIES			*100.00*	96,809	
COMB. MODAL. THERAPY			100.00	96,809	
COMB. MODAL. THERAPY - CHEM.			40.00	38,724	
COMB. MODAL. THERAPY - IMM.			60.00	58,085	
MARSHALL RESEARCH ANIMALS, INC.		N01CM60123 A.P.			36,250
PHARMACOLOGY/TOXICOLOGY			*100.00*	36,250	
ANIMALS					
MARYLAND, UNIVERSITY OF		N01CM43748 BCRC			4,081,728
PROGRAM MANAGEMENT			* 5.00*	204,086	
ADMINISTRATION					
PHASE I CLINICAL TRIALS			* 10.00*	408,173	
NON-SPECIFIC	CHEM.				
PHASE II CLINICAL TRIALS			* 34.00*	1,387,788	
LEUK.-ACUTE GRAN.	CHEM.		3.00	122,452	
LEUK.-ACUTE GRAN.	SUPPORTIVE CARE		1.00	40,817	
LEUK.-ACUTE LYMPH.	CHEM.		1.00	40,817	
LEUK.-CHRON. GRAN.	CHEM.		1.00	40,817	
LEUK.-CHRON. GRAN.	SUPPORTIVE CARE		1.00	40,817	
LEUK.-CHRON LYMPH.	CHEM.		1.00	40,817	
LYMPHOMA-HODGKIN'S	CHEM.		1.00	40,817	
LYMPHOMA-HODGKIN'S	SUPPORTIVE CARE		1.00	40,817	
LYMPHOMA-LYMPHOCYT	CHEM.		1.00	40,817	
BREAST	CHEM.		3.00	122,452	
BREAST	SUPPORTIVE CARE		1.00	40,817	
CNS TUMORS	RAD.		1.00	40,817	
CNS TUMORS	CHEM.		3.00	122,452	
CNS TUMORS	SUPPORTIVE CARE		2.00	81,635	
COLORECTAL	CHEM.		2.00	81,635	
COLORECTAL	SUPPORTIVE CARE		1.00	40,817	
KIDNEY	CHEM.		1.00	40,817	
MELANOMA	CHEM.		2.00	81,635	
OSTEOGENIC	CHEM.		3.00	122,452	
OSTEOGENIC	SUPPORTIVE CARE		1.00	40,817	

NAME		# & AREA	PERCENT OF EFFORT		DOLLAR LEVEL
NON-SPECIFIC	RAD.		2.00	81,635	
NON-SPECIFIC	CHEM.		1.00	40,817	
PHASE III CLINICAL TRIALS			* 26.00*	1,061,249	
LEUK.-ACUTE GRAN.	CHEM.		3.00	122,452	
LEUK.-ACUTE GRAN.	SUPPORTIVE CARE		1.00	40,817	
LEUK.-ACUTE LYMPH.	CHEM.		1.00	40,817	
LEUK.-ACUTE LYMPH.	SUPPORTIVE CARE		1.00	40,817	
LEUK.-CHRON. GRAN.	CHEM.		1.00	40,817	
LYMPHOMA-HODGKIN'S	CHEM.		3.00	122,452	
LYMPHOMA-HODGKIN'S	SUPPORTIVE CARE		2.00	81,635	
LYMPHOMA-LYMPHOCYT	CHEM.		2.00	81,635	
LYMPHOMA-MYCO.FUNG	CHEM.		2.00	81,635	
MYELOMA	CHEM.		2.00	81,635	
MYELOMA	SUPPORTIVE CARE		1.00	40,817	
LUNG	CHEM.		3.00	122,452	
TESTICULAR	CHEM.		2.00	81,635	
NON-SPECIFIC	CHEM.		1.00	40,817	
NON-SPECIFIC	SUPPORTIVE CARE		1.00	40,817	
PHASE IV CLINICAL TRIALS			* 25.00*	1,020,432	
LEUK.-ACUTE GRAN.	CHEM.		5.00	204,086	
LEUK.-ACUTE GRAN.	SUPPORTIVE CARE		2.00	81,635	
LEUK.-ACUTE LYMPH.	CHEM.		1.00	40,817	
LEUK.-CHRON. GRAN.	CHEM.		1.00	40,817	
LEUK.-CHRON LYMPH.	CHEM.		1.00	40,817	
LEUK.-CHRON LYMPH.	SUPPORTIVE CARE		1.00	40,817	
LYMPHOMA-HODGKIN'S	SURG.		3.00	122,452	
LYMPHOMA-HODGKIN'S	RAD.		3.00	122,452	
LYMPHOMA-HODGKIN'S	CHEM.		3.00	122,452	
LYMPHOMA-HODGKIN'S	SUPPORTIVE CARE		3.00	122,452	
LYMPHOMA-LYMPHOCYT	CHEM.		1.00	40,817	
NON-SPECIFIC	CHEM.		1.00	40,817	
MASON RESEARCH INSTITUTE		N01CM67011 C.O.P.			189,907
TREATMENT STUDIES			*100.00*	189,907	
DATA PROCESSING AND SUPPORT					
MASON RESEARCH INSTITUTE		N01CM57003 D.T.P.			696,824
ACQUISITION OF MATERIALS			* 5.00*	34,841	
BIOASSAY OF NATURAL PRODUCTS					
BASIC SCREEN			* 61.00*	425,063	
PRIMARY SCREENING, IN VIVO			50.00	348,412	
PRIMARY SCREEN., RELATED NEW MODEL DEVEL			11.00	76,651	
VERIFICATION SCREEN			* 24.00*	167,238	
DETAILED DRUG EVALUATION, IN VIVO					
TREATMENT STUDIES			* 10.00*	69,682	
COMB. MODAL. THERAPY			10.00	69,682	
COMB. MODAL. THERAPY - SURG.			8.00	55,746	
COMB. MODAL. THERAPY - CHEM.			2.00	13,936	

NAME		# & AREA	PERCENT OF EFFORT		DOLLAR LEVEL
MASON RESEARCH INSTITUTE		N01CM57030 D.T.P.			166,471
VERIFICATION SCREEN DET. DRUG EVAL., RELATED NEW MODEL DEVEL			*100.00*	166,471	
MASSACHUSETTS INSTITUTE OF		N01CM43712 D.T.P.			52,800
ACQUISITION OF MATERIALS NEW AGENT PROCUREMENT, SYNTHETICS			*100.00*	52,800	
MAYO FOUNDATION		N01CM43783 CTEP			132,141
PHASE III CLINICAL TRIALS			* 40.00*	52,856	
STOMACH	CHEM.				
PHASE IV CLINICAL TRIALS			* 45.00*	59,463	
STOMACH	SURG.		10.00	13,214	
STOMACH	RAD.		10.00	13,214	
STOMACH	CHEM.		25.00	33,035	
OTHER CLINICAL TRIALS RESEARCH			* 15.00*	19,821	
STOMACH	DATA PROC. & SUPP.				
MAYO FOUNDATION		N01CM02066 CTEP			175,000
PHASE II CLINICAL TRIALS			* 75.00*	131,250	
COLORECTAL	CHEM.		60.00	105,000	
PANCREATIC	CHEM.		7.00	12,250	
STOMACH	CHEM.		8.00	14,000	
PHASE III CLINICAL TRIALS			* 25.00*	43,750	
COLORECTAL	CHEM.		20.00	35,000	
PANCREATIC	CHEM.		3.00	5,250	
STOMACH	CHEM.		2.00	3,500	
MAYO FOUNDATION		N01CM57033 CTEP			203,590
PHASE IV CLINICAL TRIALS			* 80.00*	162,872	
COLORECTAL	SURG.		10.00	20,359	
COLORECTAL	RAD.		30.00	61,077	
COLORECTAL	CHEM.		40.00	81,436	
OTHER CLINICAL TRIALS RESEARCH			* 20.00*	40,718	
COLORECTAL	DATA PROC. & SUPP.				
MAYO FOUNDATION		N01CM57044 CTEP			507,938
PHASE II CLINICAL TRIALS			* 55.00*	279,366	
BREAST	CHEM.		15.00	76,191	
COLORECTAL	CHEM.		15.00	76,191	
HEAD AND NECK	CHEM.		5.00	25,397	
LUNG	CHEM.		5.00	25,397	
MELANOMA	CHEM.		10.00	50,794	
SARCOMAS (GEN.)	CHEM.		5.00	25,397	

NAME		# & AREA	PERCENT OF EFFORT		DOLLAR LEVEL
PHASE III CLINICAL TRIALS			* 45.00*	228,572	
BREAST	CHEM.		10.00	50,794	
COLORECTAL	CHEM.		5.00	25,397	
HEAD AND NECK	CHEM.		5.00	25,397	
LUNG	CHEM.		5.00	25,397	
MELANOMA	CHEM.		15.00	76,191	
SARCOMAS (GEN.)	CHEM.		5.00	25,397	
MAYO FOUNDATION		N01CM53772 CTEP			121,385
PHASE II CLINICAL TRIALS			*100.00*	121,385	
BREAST	CHEM.				
MAYO FOUNDATION		N01CM53838 CTEP			80,292
PHASE I CLINICAL TRIALS			* 80.00*	64,234	
NON-SPECIFIC	CHEM.		50.00	40,146	
NON-SPECIFIC	PHARM./TOX.		30.00	24,088	
OTHER CLINICAL TRIALS RESEARCH			* 20.00*	16,058	
NON-SPECIFIC	PROTOCOL-REL. STAT		5.00	4,015	
NON-SPECIFIC	DATA PROC. & SUPP.		15.00	12,044	
MAYO FOUNDATION		N01CM43796 CTEP			211,093
PHASE II CLINICAL TRIALS			* 30.00*	63,328	
PANCREATIC	CHEM.				
PHASE IV CLINICAL TRIALS			* 50.00*	105,547	
PANCREATIC	SURG.		5.00	10,555	
PANCREATIC	RAD.		20.00	42,219	
PANCREATIC	CHEM.		25.00	52,773	
OTHER CLINICAL TRIALS RESEARCH			* 20.00*	42,219	
PANCREATIC	DATA PROC. & SUPP.				
MAYO FOUNDATION		N01CM67123 CTEP			214,252
PHASE III CLINICAL TRIALS			* 50.00*	107,126	
OVARIAN	SURG.		15.00	32,138	
OVARIAN	RAD.		15.00	32,138	
OVARIAN	CHEM.		20.00	42,850	
PHASE IV CLINICAL TRIALS			* 50.00*	107,126	
OVARIAN	SURG.		15.00	32,138	
OVARIAN	RAD.		15.00	32,138	
OVARIAN	CHEM.		20.00	42,850	
MAYO FOUNDATION		N01CM77150 CTEP			175,640
PHASE II CLINICAL TRIALS			* 20.00*	35,128	
LUNG	RAD.		10.00	17,564	
LUNG	CHEM.		10.00	17,564	
PHASE III CLINICAL TRIALS			* 60.00*	105,384	
LUNG	RAD.		30.00	52,692	

NAME		# & AREA	PERCENT OF EFFORT		DOLLAR LEVEL
LUNG	CHEM.		30.00	52,692	
PHASE IV CLINICAL TRIALS			* 20.00*	35,128	
LUNG	RAD.		10.00	17,564	
LUNG	CHEM.		10.00	17,564	
MEMORIAL HOSP. FOR CANCER & ALLIED		N01CM57019 BCRC			139,386
PROGRAM MANAGEMENT ADMINISTRATION			* 10.00*	13,939	
PHASE II CLINICAL TRIALS			* 30.00*	41,816	
CNS TUMORS	RAD.				
PHASE III CLINICAL TRIALS			* 60.00*	83,632	
CNS TUMORS	RAD.		30.00	41,816	
CNS TUMORS	CHEM.		30.00	41,816	
MEMORIAL HOSP. FOR CANCER & ALLIED		N01CM57043 CTEP			438,072
PHASE II CLINICAL TRIALS			* 60.00*	262,843	
BREAST	CHEM.		5.00	21,904	
CNS TUMORS	CHEM.		5.00	21,904	
HEAD AND NECK	CHEM.		5.00	21,904	
LUNG	CHEM.		20.00	87,614	
MELANOMA	CHEM.		5.00	21,904	
SARCOMAS (GEN.)	CHEM.		10.00	43,807	
STOMACH	CHEM.		5.00	21,904	
TESTICULAR	CHEM.		5.00	21,904	
PHASE III CLINICAL TRIALS			* 40.00*	175,229	
BREAST	CHEM.		5.00	21,904	
COLORECTAL	CHEM.		5.00	21,904	
HEAD AND NECK	CHEM.		5.00	21,904	
LUNG	CHEM.		5.00	21,904	
MELANOMA	CHEM.		5.00	21,904	
STOMACH	CHEM.		5.00	21,904	
TESTICULAR	CHEM.		10.00	43,807	
MEMORIAL HOSP. FOR CANCER & ALLIED		N01CP65829 CTEP			31,979
CLINICAL TRIALS SUPPORTIVE RESEARCH			*100.00*	31,979	
HEAD AND NECK	NUTRITION		20.00	6,396	
LUNG	NUTRITION		20.00	6,396	
OVARIAN	NUTRITION		20.00	6,396	
PANCREATIC	NUTRITION		20.00	6,396	
STOMACH	NUTRITION		20.00	6,396	
MEMORIAL HOSP. FOR CANCER & ALLIED		N01CM43804 CTEP			140,408
PHASE I CLINICAL TRIALS			*100.00*	140,408	
NON-SPECIFIC	CHEM.				

NAME		# & AREA	PERCENT OF EFFORT		DOLLAR LEVEL
MEMORIAL HOSP. FOR CANCER & ALLIED		N01CM53844 CTEP			113,116
PHASE II CLINICAL TRIALS			* 16.00*	18,099	
COLORECTAL	RAD.		3.00	3,393	
COLORECTAL	CHEM.		10.00	11,312	
COLORECTAL	IMM.		3.00	3,393	
PHASE III CLINICAL TRIALS			* 84.00*	95,017	
COLORECTAL	RAD.		17.00	19,230	
COLORECTAL	CHEM.		50.00	56,558	
COLORECTAL	IMM.		17.00	19,230	
MIAMI, UNIVERSITY OF		N01CM43800 CTEP			163,119
PHASE II CLINICAL TRIALS			* 35.00*	57,092	
PANCREATIC	SURG.		1.75	2,855	
PANCREATIC	CHEM.		21.00	34,255	
PANCREATIC	IMM.		12.25	19,982	
PHASE III CLINICAL TRIALS			* 55.00*	89,715	
PANCREATIC	SURG.		5.50	8,972	
PANCREATIC	RAD.		19.25	31,400	
PANCREATIC	CHEM.		19.25	31,400	
PANCREATIC	IMM.		11.00	17,943	
PHASE IV CLINICAL TRIALS			* 10.00*	16,312	
PANCREATIC	SURG.		1.00	1,631	
PANCREATIC	RAD.		2.00	3,262	
PANCREATIC	CHEM.		5.00	8,156	
PANCREATIC	IMM.		2.00	3,262	
MIAMI, UNIVERSITY OF		N01CM67093 CTEP			106,843
PHASE IV CLINICAL TRIALS			*100.00*	106,843	
COLORECTAL	SURG.		20.00	21,369	
COLORECTAL	RAD.		15.00	16,026	
COLORECTAL	CHEM.		45.00	48,079	
COLORECTAL	IMM.		20.00	21,369	
MIAMI, UNIVERSITY OF		N01CM67077 D.T.P.			137,508
ACQUISITION OF MATERIALS			* 5.00*	6,875	
BIOASSAY OF NATURAL PRODUCTS					
BASIC SCREEN			* 95.00*	130,633	
PRIMARY SCREENING, IN VITRO			90.00	123,757	
PRIMARY SCREEN., RELATED NEW MODEL DEVEL			5.00	6,875	
MIAMI, UNIVERSITY OF		N01CM57041 D.T.P.			106,157
BIOLOGICAL STUDIES			*100.00*	106,157	
IMMUNOBIOLOGY			40.00	42,463	
IMMUNOSUPPRESSION			60.00	63,694	

NAME	# & AREA	PERCENT OF EFFORT		DOLLAR LEVEL
MICHIGAN CANCER FOUNDATION	N01CM53789 D.T.P.			36,450
VERIFICATION SCREEN		*100.00*	36,450	
DETAILED DRUG EVALUATION, IN VITRO		80.00	29,160	
DET. DRUG EVAL., RELATED NEW MODEL DEVEL		20.00	7,290	
MICHIGAN DEPARTMENT OF PUBLIC HEALTH	N01CM33702 D.T.P.			124,426
BASIC SCREEN		*100.00*	124,426	
PRIMARY SCREENING, IN VITRO				
MICROBIAL CHEMISTRY RESEARCH FDN.	N01CM57009 D.T.P.			75,000
ACQUISITION OF MATERIALS		* 60.00*	45,000	
NEW AGENT PROCUREMENT, FERMENT/ANTIBIOT				
BASIC SCREEN		* 40.00*	30,000	
PRIMARY SCREEN., RELATED NEW MODEL DEVEL				
MICROBIOLOGICAL ASSOCIATES, INC.	N01CM67069 A.P.			187,600
ACQUISITION OF MATERIALS		* 20.00*	37,520	
ANIMALS				
BASIC SCREEN		* 55.00*	103,180	
ANIMALS				
VERIFICATION SCREEN		* 25.00*	46,900	
ANIMALS				
MICROBIOLOGICAL ASSOCIATES, INC.	N01CM53818 A.P.			250,000
ACQUISITION OF MATERIALS		* 20.00*	50,000	
ANIMALS				
BASIC SCREEN		* 55.00*	137,500	
ANIMALS				
VERIFICATION SCREEN		* 25.00*	62,500	
ANIMALS				
MICROBIOLOGICAL ASSOCIATES, INC.	N01CM77131 BCRC			92,900
PROGRAM MANAGEMENT		* 7.00*	6,503	
ADMINISTRATION		2.00	1,858	
DATA PROCESSING AND SUPPORT		5.00	4,645	
CLINICAL TRIALS SUPPORTIVE RESEARCH		* 93.00*	86,397	
LEUK.-ACUTE GRAN. VIROLOGY		30.00	27,870	
LEUK.-ACUTE LYMPH. VIROLOGY		5.00	4,645	
LEUK.-CHRON. GRAN. VIROLOGY		10.00	9,290	
LEUK.-CHRON LYMPH. VIROLOGY		5.00	4,645	
LYMPHOMA-HODGKIN'S VIROLOGY		20.00	18,580	
LYMPHOMA-LYMPHOCYT VIROLOGY		5.00	4,645	
LYMPHOMA-MYCO.FUNG VIROLOGY		3.00	2,787	
MYELOMA VIROLOGY		5.00	4,645	

NAME		# & AREA	PERCENT OF EFFORT		DOLLAR LEVEL
CNS TUMORS	VIROLOGY		5.00	4,645	
MELANOMA	VIROLOGY		5.00	4,645	
MICROBIOLOGICAL ASSOCIATES, INC.		N01CM63810 C.O.P.			200,000
BIOLOGICAL STUDIES			*100.00*	200,000	
IMMUNOBIOLOGY			50.00	100,000	
SERUM STORAGE			50.00	100,000	
MICROBIOLOGICAL ASSOCIATES, INC.		N01CM43793 D.T.P.			464,000
ACQUISITION OF MATERIALS			* 60.00*	278,400	
DATA PROCESSING AND SUPPORT			10.00	46,400	
STORAGE DISTRIBUTION			50.00	232,000	
PROCUREMENT OF PRECLINICAL MATERIAL			* 30.00*	139,200	
STORAGE DISTRIBUTION					
PROD. AND FORM. FOR CLINICAL TRIALS			* 10.00*	46,400	
DATA PROCESSING AND SUPPORT			1.00	4,640	
STORAGE DISTRIBUTION			9.00	41,760	
MIDWEST RESEARCH INSTITUTE		N01CM33722 D.T.P.			228,823
PROCUREMENT OF PRECLINICAL MATERIAL			* 20.00*	45,765	
ANALYTICAL AND QUALITY CONTROL					
PROD. AND FORM. FOR CLINICAL TRIALS			* 80.00*	183,058	
ANALYTICAL AND QUALITY CONTROL					
MIDWEST RESEARCH INSTITUTE		N01CM33743 D.T.P.			395,365
ACQUISITION OF MATERIALS			* 80.00*	316,292	
NEW AGENT PROCUREMENT, SYNTHETICS					
BASIC SCREEN			* 6.00*	23,722	
PRIMARY SCREENING, IN VITRO					
PROCUREMENT OF PRECLINICAL MATERIAL			* 14.00*	55,351	
SYNTHETICS					
MINNESOTA, UNIVERSITY OF		N01CM67113 C.O.P.			54,640
CLINICAL TRIALS SUPPORTIVE RESEARCH			*100.00*	54,640	
LYMPHOMA-HISTIOCYT	PATHOLOGIC CLASSIF		50.00	27,320	
LYMPHOMA-LYMPHOCYT	PATHOLOGIC CLASSIF		50.00	27,320	
MINNESOTA, UNIVERSITY OF		N01CM43788 D.T.P.			53,100
VERIFICATION SCREEN			*100.00*	53,100	
DETAILED DRUG EVALUATION, IN VITRO			80.00	42,480	
DET. DRUG EVAL., RELATED NEW MODEL DEVEL			20.00	10,620	
MINNESOTA, UNIVERSITY OF		N01HB62972 O.D.			134,528
CLINICAL TRIALS SUPPORTIVE RESEARCH			*100.00*	134,528	

NAME		# & AREA	PERCENT OF EFFORT		DOLLAR LEVEL
OTHER	TRANSFUSION RES.				
MISSOURI, UNIVERSITY OF		N01CM12323 D.T.P.			210,000
CLINICAL TRIALS SUPPORTIVE RESEARCH			*100.00*	210,000	
NON-SPECIFIC	MARKERS				
MONSANTO RESEARCH CORPORATION		N01CM67046 D.T.P.			58,500
PROCUREMENT OF PRECLINICAL MATERIAL			* 10.00*	5,850	
PLANT PRODUCTS					
PROD. AND FORM. FOR CLINICAL TRIALS			* 90.00*	52,650	
PRODUCTION, SYNTHETICS			15.00	8,775	
PRODUCTION, PLANT PRODUCTS			75.00	43,875	
MONSANTO RESEARCH CORPORATION		N01CM77140 D.T.P.			478,137
PROD. AND FORM. FOR CLINICAL TRIALS			*100.00*	478,137	
PRODUCTION, PLANT PRODUCTS					
MONTEFIORE HOSPITAL		N01CM67061 BCRC			88,289
PROGRAM MANAGEMENT			* 10.00*	8,829	
ADMINISTRATION					
PHASE II CLINICAL TRIALS			* 30.00*	26,487	
CNS TUMORS	RAD.				
PHASE III CLINICAL TRIALS			* 60.00*	52,973	
CNS TUMORS	RAD.		30.00	26,487	
CNS TUMORS	CHEM.		30.00	26,487	
MOUNT SINAI SCHOOL OF MEDICINE		N01CM53837 CTEP			117,190
PHASE I CLINICAL TRIALS			* 80.00*	93,752	
NON-SPECIFIC	CHEM.		50.00	58,595	
NON-SPECIFIC	PHARM./TOX.		30.00	35,157	
OTHER CLINICAL TRIALS RESEARCH			* 20.00*	23,438	
NON-SPECIFIC	PROTOCOL-REL. STAT		5.00	5,860	
NON-SPECIFIC	DATA PROC. & SUPP.		15.00	17,579	
MOUNT SINAI SCHOOL OF MEDICINE		N01CM53841 CTEP			192,027
PHASE IV CLINICAL TRIALS			* 90.00*	172,824	
STOMACH	SURG.		25.00	48,007	
STOMACH	RAD.		25.00	48,007	
STOMACH	CHEM.		40.00	76,811	
CLINICAL TRIALS SUPPORTIVE RESEARCH			* 10.00*	19,203	
STOMACH	MARKERS				
MOUNT SINAI SCHOOL OF MEDICINE		N01CM43703 CTEP			49,492
PHASE III CLINICAL TRIALS			*100.00*	49,492	

NAME		# & AREA	PERCENT OF EFFORT		DOLLAR LEVEL
OVARIAN	CHEM.				
MOUNT SINAI SCHOOL OF MEDICINE		N01CM67096 CTEP			120,246
PHASE IV CLINICAL TRIALS			* 90.00*	108,221	
COLORECTAL	SURG.		18.00	21,644	
COLORECTAL	RAD.		18.00	21,644	
COLORECTAL	CHEM.		36.00	43,289	
COLORECTAL	IMM.		18.00	21,644	
CLINICAL TRIALS SUPPORTIVE RESEARCH			* 10.00*	12,025	
COLORECTAL	MARKERS				
MURPHY BREEDING LABS., INC.		N01CM50579 A.P.			212,160
ACQUISITION OF MATERIALS ANIMALS			* 20.00*	42,432	
BASIC SCREEN ANIMALS			* 55.00*	116,688	
VERIFICATION SCREEN ANIMALS			* 25.00*	53,040	
NATIONAL ACADEMY OF SCIENCES		N01CM57013 A.P.			24,000
PROGRAM MANAGEMENT COMMUNICATION AND EDUCATION			*100.00*	24,000	
NATIONAL ACADEMY OF SCIENCES		N01CM53850 A.P.			20,000
PROGRAM MANAGEMENT COMMUNICATION AND EDUCATION			*100.00*	20,000	
NATIONAL NAVAL MEDICAL CENTER		Y01CM50100 CTEP			31,600
PHASE III CLINICAL TRIALS			*100.00*	31,600	
BREAST	CHEM.				
NAVAL WEAPONS CENTER		Y01CM50101 O.D.			34,000
BIOLOGICAL STUDIES BLOOD PRODUCTS			*100.00*	34,000	
NEW ENGLAND MEDICAL CENTER		N01CM67112 C.O.P.			62,679
CLINICAL TRIALS SUPPORTIVE RESEARCH			*100.00*	62,679	
LYMPHOMA-HISTIOCYT	PATHOLOGIC CLASSIF		50.00	31,340	
LYMPHOMA-LYMPHOCYT	PATHOLOGIC CLASSIF		50.00	31,340	
NEW YORK UNIVERSITY MEDICAL CENTER		N01CM67059 BCRC			72,462
PROGRAM MANAGEMENT ADMINISTRATION			* 10.00*	7,246	

NAME		# & AREA	PERCENT OF EFFORT		DOLLAR LEVEL
PHASE II CLINICAL TRIALS			* 30.00*	21,739	
CNS TUMORS	CHEM.				
PHASE III CLINICAL TRIALS			* 60.00*	43,477	
CNS TUMORS	RAD.		30.00	21,739	
CNS TUMORS	CHEM.		30.00	21,739	
NORTHEASTERN UNIVERSITY		N01CM53741 D.T.P.			72,361
ACQUISITION OF MATERIALS			* 95.00*	68,743	
NEW AGENT PROCUREMENT, SYNTHETICS					
PROCUREMENT OF PRECLINICAL MATERIAL			* 5.00*	3,618	
SYNTHETICS					
OHIO STATE UNIVERSITY RESEARCH		N01CM67060 BCRC			28,123
PROGRAM MANAGEMENT			* 10.00*	2,812	
ADMINISTRATION					
PHASE II CLINICAL TRIALS			* 30.00*	8,437	
CNS TUMORS	CHEM.				
PHASE III CLINICAL TRIALS			* 60.00*	16,874	
CNS TUMORS	RAD.		30.00	8,437	
CNS TUMORS	CHEM.		30.00	8,437	
OHIO STATE UNIVERSITY RESEARCH		N01CM33735 CTEP			31,895
PHASE II CLINICAL TRIALS			* 20.00*	6,379	
LUNG	RAD.		10.00	3,190	
LUNG	CHEM.		10.00	3,190	
PHASE III CLINICAL TRIALS			* 60.00*	19,137	
LUNG	RAD.		30.00	9,569	
LUNG	CHEM.		30.00	9,569	
PHASE IV CLINICAL TRIALS			* 20.00*	6,379	
LUNG	RAD.		10.00	3,190	
LUNG	CHEM.		10.00	3,190	
OHIO STATE UNIVERSITY RESEARCH		N01CM57015 D.T.P.			68,223
ACQUISITION OF MATERIALS			* 90.00*	61,401	
NEW AGENT PROCUREMENT, SYNTHETICS					
PROCUREMENT OF PRECLINICAL MATERIAL			* 10.00*	6,822	
SYNTHETICS					
OHIO STATE UNIVERSITY RESEARCH		N01CM53828 D.T.P.			117,258
PHARMACOLOGY/TOXICOLOGY			* 10.00*	11,726	
PHARMACOLOGY					
PHASE I CLINICAL TRIALS			* 80.00*	93,806	
NON-SPECIFIC	PHARM./TOX.				
PHASE II CLINICAL TRIALS			* 10.00*	11,726	
NON-SPECIFIC	PHARM./TOX.				

NAME	# & AREA	PERCENT OF EFFORT		DOLLAR LEVEL
OKLAHOMA, UNIVERSITY OF	N01CM57108 D.T.P.			59,203
ACQUISITION OF MATERIALS		*100.00*	59,203	
NEW AGENT PROCUREMENT, MARINE/ARTHROPODS				
ONTARIO CANCER INSTITUTE	N01CM77152 CTEP			146,766
PHASE II CLINICAL TRIALS		* 20.00*	29,353	
LUNG RAD.		10.00	14,677	
LUNG CHEM.		10.00	14,677	
PHASE III CLINICAL TRIALS		* 60.00*	88,060	
LUNG RAD.		30.00	44,030	
LUNG CHEM.		30.00	44,030	
PHASE IV CLINICAL TRIALS		* 20.00*	29,353	
LUNG RAD.		10.00	14,677	
LUNG CHEM.		10.00	14,677	
OREGON STATE UNIVERSITY	N01CM23201 D.T.P.			92,840
PHARMACOLOGY/TOXICOLOGY		*100.00*	92,840	
PHARMACOLOGY				
PARKE, DAVIS AND COMPANY	N01CM43754 D.T.P.			129,749
ACQUISITION OF MATERIALS		* 85.00*	110,287	
NEW AGENT PROCUREMENT, FERMENT/ANTIBIOT		83.00	107,692	
DATA PROCESSING AND SUPPORT		2.00	2,595	
BASIC SCREEN		* 15.00*	19,462	
PRIMARY SCREENING, IN VITRO		10.00	12,975	
PRIMARY SCREEN., RELATED NEW MODEL DEVEL		5.00	6,487	
PARKE, DAVIS AND COMPANY	N01CM77146 D.T.P.			664,642
ACQUISITION OF MATERIALS		* 85.00*	564,946	
NEW AGENT PROCUREMENT, FERMENT/ANTIBIOT		83.00	551,653	
DATA PROCESSING AND SUPPORT		2.00	13,293	
BASIC SCREEN		* 15.00*	99,696	
PRIMARY SCREENING, IN VITRO		10.00	66,464	
PRIMARY SCREEN., RELATED NEW MODEL DEVEL		5.00	33,232	
PARKE, DAVIS AND COMPANY	N01CM23708 D.T.P.			189,385
PROCUREMENT OF PRECLINICAL MATERIAL		* 50.00*	94,693	
SYNTHETICS				
PROD. AND FORM. FOR CLINICAL TRIALS		* 50.00*	94,693	
PRODUCTION, SYNTHETICS		25.00	47,346	
PRODUCTION, PLANT PRODUCTS		25.00	47,346	

NAME	# & AREA	PERCENT OF EFFORT		DOLLAR LEVEL
PENNSYLVANIA, UNIVERSITY OF	N01CM53830 D.T.P.			34,530
VERIFICATION SCREEN		*100.00*	34,530	
DETAILED DRUG EVALUATION, IN VITRO		50.00	17,265	
DET. DRUG EVAL., RELATED NEW MODEL DEVEL		50.00	17,265	
PFIZER, INC.	N01CM53805 D.T.P.			289,000
ACQUISITION OF MATERIALS		*100.00*	289,000	
ANIMAL VIRUSES				
PHARM-ECO	N01CM23707 D.T.P.			141,093
PROCUREMENT OF PRECLINICAL MATERIAL		* 30.00*	42,328	
SYNTHETICS				
PROD. AND FORM. FOR CLINICAL TRIALS		* 70.00*	98,765	
PRODUCTION, SYNTHETICS				
PHILIPS ROXANE LABORATORIES, INC.	N01CM67053 D.T.P.			126,921
FORMULATION		* 20.00*	25,384	
DEVEL. OF EXP. FORMULATIONS				
PROD. AND FORM. FOR CLINICAL TRIALS		* 80.00*	101,537	
FORMULATION		70.00	88,845	
ANALYTICAL AND QUALITY CONTROL		10.00	12,692	
POLYSCIENCES, INC.	N01CM77070 D.T.P.			280,845
ACQUISITION OF MATERIALS		*100.00*	280,845	
NEW AGENT PROCUREMENT, PLANT PRODUCTS		79.00	221,868	
NEW AGENT PROCURFMENT, FERMENT/ANTIBIOT		10.00	28,085	
BIOASSAY OF NATURAL PRODUCTS		11.00	30,893	
POMONA COLLEGE	N01CM67062 D.T.P.			53,000
ACQUISITION OF MATERIALS		*100.00*	53,000	
STRUCTURE ACTIVITY				
PUBLIC HEALTH SERVICE	Y01CM40003 BCRC			139,815
PHARMACOLOGY/TOXICOLOGY		* 35.00*	48,935	
PHARMACOLOGY		30.00	41,945	
DATA PROCESSING AND SUPPORT		5.00	6,991	
BIOLOGICAL STUDIES		* 55.00*	76,898	
BIOCHEMISTRY		10.00	13,982	
CELL BIOLOGY		10.00	13,982	
CELL KINETICS		5.00	6,991	
IMMUNOBIOLOGY		10.00	13,982	
MARKERS		5.00	6,991	
MOLECULAR BIOLOGY		15.00	20,972	

NAME	# & AREA	PERCENT OF EFFORT		DOLLAR LEVEL
PROGRAM MANAGEMENT ADMINISTRATION		* 10.00*	13,982	
PURDUE UNIVERSITY	N01CM67091 D.T.P.			125,773
ACQUISITION OF MATERIALS		* 85.00*	106,907	
NEW AGENT PROCUREMENT, PLANT PRODUCTS		65.00	81,752	
NEW AGENT PROCUREMENT, FERMENT/ANTIBIOT		15.00	18,866	
BIOASSAY OF NATURAL PRODUCTS		5.00	6,289	
PROCUREMENT OF PRECLINICAL MATERIAL		* 15.00*	18,866	
PLANT PRODUCTS		10.00	12,577	
FERMENTATION/ANTIBIOTICS		5.00	6,289	
RADIOBIOLOGICAL INST., ORGAN. FOR	N01CM53753 D.T.P.			73,151
TREATMENT STUDIES		*100.00*	73,151	
COMB. MODAL. THERAPY		100.00	73,151	
COMB. MODAL. THERAPY - RAD.		50.00	36,576	
COMB. MODAL. THERAPY - CHEM.		50.00	36,576	
RES. FDN. OF STATE UNIVERSITY OF NEW	N01CM33715 CTEP			192,424
CLINICAL TRIALS SUPPORTIVE RESEARCH		*100.00*	192,424	
LUNG	DATA PROC. & SUPP.			
RES. FDN. OF STATE UNIVERSITY OF NEW	N01CM53766 D.T.P.			151,164
BIOLOGICAL STUDIES		*100.00*	151,164	
IMMUNOBIOLOGY		40.00	60,466	
IMMUNOSUPPRESSION		60.00	90,698	
RESEARCH TRIANGLE INSTITUTE	N01CM67121 D.T.P.			235,000
PROCUREMENT OF PRECLINICAL MATERIAL RADIOLABELED MATERIALS		* 80.00*	188,000	
PROD. AND FORM. FOR CLINICAL TRIALS RADIOLABEL		* 20.00*	47,000	
RESEARCH TRIANGLE INSTITUTE	N01CM67089 D.T.P.			190,174
ACQUISITION OF MATERIALS		* 85.00*	161,648	
NEW AGENT PROCUREMENT, PLANT PRODUCTS		65.00	123,613	
NEW AGENT PROCUREMENT, FERMENT/ANTIBIOT		15.00	28,526	
BIOASSAY OF NATURAL PRODUCTS		5.00	9,509	
PROCUREMENT OF PRECLINICAL MATERIAL		* 15.00*	28,526	
PLANT PRODUCTS		10.00	19,017	
FERMENTATION/ANTIBIOTICS		5.00	9,509	
RUTGERS STATE UNIVERSITY	N01CM67106 D.T.P.			98,192
ACQUISITION OF MATERIALS		*100.00*	98,192	

NAME		# & AREA	PERCENT OF EFFORT		DOLLAR LEVEL
NEW AGENT PROCUREMENT, FERMENT/ANTIBIOT					
SAINT JUDE'S CHILDREN'S RESEARCH		N01CM77127 D.T.P.			89,145
ACQUISITION OF MATERIALS			*100.00*	89,145	
NEW AGENT PROCUREMENT, SYNTHETICS					
SAINT LOUIS UNIVERSITY SCHOOL OF		N01CM57020 BCRC			122,547
PROGRAM MANAGEMENT			* 10.00*	12,255	
ADMINISTRATION					
PHASE II CLINICAL TRIALS			* 30.00*	36,764	
CNS TUMORS	CHEM.				
PHASE III CLINICAL TRIALS			* 60.00*	73,528	
CNS TUMORS	RAD.		30.00	36,764	
CNS TUMORS	CHEM.		30.00	36,764	
SIDNEY FARBER CANCER INSTITUTE		N01CM57035 CTEP			145,942
PHASE IV CLINICAL TRIALS			*100.00*	145,942	
COLORECTAL	RAD.		3.00	4,378	
COLORECTAL	CHEM.		35.00	51,080	
COLORECTAL	IMM.		22.00	32,107	
COLORECTAL	SUPPORTIVE CARE		40.00	58,377	
SIDNEY FARBER CANCER INSTITUTE		N01CM43781 CTEP			18,672
PHASE IV CLINICAL TRIALS			*100.00*	18,672	
STOMACH	RAD.		15.00	2,801	
STOMACH	CHEM.		78.00	14,564	
STOMACH	SUPPORTIVE CARE		7.00	1,307	
SIDNEY FARBER CANCER INSTITUTE		N01CM67037 CTEP			406,768
PHASE II CLINICAL TRIALS			* 55.00*	223,722	
BREAST	CHEM.		15.00	61,015	
COLORECTAL	CHEM.		15.00	61,015	
HEAD AND NECK	CHEM.		5.00	20,338	
LUNG	CHEM.		5.00	20,338	
MELANOMA	CHEM.		10.00	40,677	
SARCOMAS (GEN.)	CHEM.		5.00	20,338	
PHASE III CLINICAL TRIALS			* 45.00*	183,046	
BREAST	CHEM.		10.00	40,677	
COLORECTAL	CHEM.		5.00	20,338	
HEAD AND NECK	CHEM.		5.00	20,338	
LUNG	CHEM.		5.00	20,338	
MELANOMA	CHEM.		15.00	61,015	
SARCOMAS (GEN.)	CHEM.		5.00	20,338	

NAME		# & AREA	PERCENT OF EFFORT		DOLLAR LEVEL
SIDNEY FARBER CANCER INSTITUTE		N01CM53839 CTEP			88,983
PHASE I CLINICAL TRIALS			*100.00*	88,983	
BREAST	CHEM.		10.00	8,898	
COLORECTAL	CHEM.		10.00	8,898	
HEAD AND NECK	CHEM.		10.00	8,898	
KIDNEY	CHEM.		10.00	8,898	
LUNG	CHEM.		10.00	8,898	
MELANOMA	CHEM.		15.00	13,347	
PANCREATIC	CHEM.		10.00	8,898	
SARCOMAS (GEN.)	CHEM.		10.00	8,898	
STOMACH	CHEM.		10.00	8,898	
NON-SPECIFIC	CHEM.		5.00	4,449	
SIMONSEN LABORATORIES		N01CM53847 A.P.			165,000
ACQUISITION OF MATERIALS ANIMALS			* 20.00*	33,000	
BASIC SCREEN ANIMALS			* 55.00*	90,750	
VERIFICATION SCREEN ANIMALS			* 25.00*	41,250	
SIMONSEN LABORATORIES		N01CM50578 A.P.			224,640
ACQUISITION OF MATERIALS ANIMALS			* 20.00*	44,928	
BASIC SCREEN ANIMALS			* 55.00*	123,552	
VERIFICATION SCREEN ANIMALS			* 25.00*	56,160	
SIMONSEN LABORATORIES		N01CM53817 A.P.			600,000
ACQUISITION OF MATERIALS ANIMALS			* 20.00*	120,000	
BASIC SCREEN ANIMALS			* 55.00*	330,000	
VERIFICATION SCREEN ANIMALS			* 25.00*	150,000	
SLOAN-KETTERING INSTITUTE FOR CANCER		N01CM53820 D.T.P.			82,366
PROCUREMENT OF PRECLINICAL MATERIAL RADIOLABELED MATERIALS			* 25.00*	20,592	
BIOLOGICAL STUDIES MOLECULAR BIOLOGY			* 75.00*	61,775	

NAME		# & AREA	PERCENT OF EFFORT		DOLLAR LEVEL
SMALL BUSINESS ADMINISTRATION		N01CM43719			150,485
		D.T.P.			
ACQUISITION OF MATERIALS			* 70.00*	105,340	
ANIMAL CELLS					
BIOLOGICAL STUDIES			* 30.00*	45,146	
MOLECULAR BIOLOGY			10.00	15,049	
RADIOIMMUNE ASSAY			20.00	30,097	
SOUTHERN ANIMAL FARMS		N01CM50599			219,960
		A.P.			
ACQUISITION OF MATERIALS			* 20.00*	43,992	
ANIMALS					
BASIC SCREEN			* 55.00*	120,978	
ANIMALS					
VERIFICATION SCREEN			* 25.00*	54,990	
ANIMALS					
SOUTHERN ANIMAL FARMS		N01CM53845			125,000
		A.P.			
ACQUISITION OF MATERIALS			* 20.00*	25,000	
ANIMALS					
BASIC SCREEN			* 55.00*	68,750	
ANIMALS					
VERIFICATION SCREEN			* 25.00*	31,250	
ANIMALS					
SOUTHERN CALIFORNIA, UNIVERSITY OF		N01CM53842			20,085
		CTEP			
PHASE IV CLINICAL TRIALS			* 90.00*	18,077	
STOMACH	SURG.		25.00	5,021	
STOMACH	RAD.		25.00	5,021	
STOMACH	CHEM.		40.00	8,034	
CLINICAL TRIALS SUPPORTIVE RESEARCH			* 10.00*	2,009	
STOMACH	MARKERS				
SOUTHERN CALIFORNIA, UNIVERSITY OF		N01CM67065			130,000
		D.T.P.			
PHARMACOLOGY/TOXICOLOGY			* 75.00*	97,500	
PHARMACOLOGY			65.00	84,500	
DATA PROCESSING AND SUPPORT			10.00	13,000	
CLINICAL TRIALS SUPPORTIVE RESEARCH			* 25.00*	32,500	
NON-SPECIFIC	SPEC. PHARM./TOX.		20.00	26,000	
NON-SPECIFIC	DATA PROC. & SUPP.		5.00	6,500	
SOUTHERN CALIFORNIA, UNIVERSITY OF		N01CM67084			131,038
		D.T.P.			
ACQUISITION OF MATERIALS			*100.00*	131,038	
NEW AGENT PROCUREMENT, SYNTHETICS					

NAME	# & AREA	PERCENT OF EFFORT		DOLLAR LEVEL
SOUTHERN RESEARCH INSTITUTE	N01CM43784 D.T.P.			300,603
VERIFICATION SCREEN		*100.00*	300,603	
DETAILED DRUG EVALUATION, IN VITRO		60.00	180,362	
DETAILED DRUG EVALUATION, IN VIVO		20.00	60,121	
DET. DRUG EVAL., RELATED NEW MODEL DEVEL		20.00	60,121	
SOUTHERN RESEARCH INSTITUTE	N01CM43780 D.T.P.			215,000
PHARMACOLOGY/TOXICOLOGY		*100.00*	215,000	
PHARMACOLOGY				
SOUTHERN RESEARCH INSTITUTE	N01CM43762 D.T.P.			691,991
ACQUISITION OF MATERIALS		* 80.00*	553,593	
NEW AGENT PROCUREMENT, SYNTHETICS				
PROCUREMENT OF PRECLINICAL MATERIAL		* 20.00*	138,398	
SYNTHETICS				
SOUTHERN RESEARCH INSTITUTE	N01CM43756 D.T.P.			2,288,000
ACQUISITION OF MATERIALS		* 1.00*	22,880	
BIOASSAY OF NATURAL PRODUCTS				
BASIC SCREEN		* 32.00*	732,160	
PRIMARY SCREENING, IN VITRO		4.00	91,520	
PRIMARY SCREENING, IN VIVO		16.00	366,080	
PRIMARY SCREEN., RELATED NEW MODEL DEVEL		10.00	228,800	
ANALOG SCREENING		2.00	45,760	
VERIFICATION SCREEN		* 26.00*	594,880	
DETAILED DRUG EVALUATION, IN VIVO		14.00	320,320	
DET. DRUG EVAL., RELATED NEW MODEL DEVEL		10.00	228,800	
ANTIVIRAL ACTIVITY		2.00	45,760	
PHARMACOLOGY/TOXICOLOGY		* 2.00*	45,760	
SPECIAL TOXICITY STUDIES		1.00	22,880	
BIOASSAY-DRUG METAB.		1.00	22,880	
BIOLOGICAL STUDIES		* 1.00*	22,880	
CELL KINETICS				
TREATMENT STUDIES		* 38.00*	869,440	
COMB. MODAL. THERAPY		38.00	869,440	
COMB. MODAL. THERAPY - SURG.		17.00	388,960	
COMB. MODAL. THERAPY - CHEM.		21.00	480,480	
SOUTHERN RESEARCH INSTITUTE	N01CM57000 D.T.P.			337,290
PHARMACOLOGY/TOXICOLOGY		* 70.00*	236,103	
SPECIAL TOXICITY STUDIES				
TREATMENT STUDIES		* 30.00*	101,187	
CHEMOTHERAPY				

NAME		# & AREA	PERCENT OF EFFORT		DOLLAR LEVEL
SOUTHWEST FOUNDATION FOR RESEARCH &		N01CM67075 D.T.P.			106,153
ACQUISITION OF MATERIALS			* 5.00*	5,308	
BIOASSAY OF NATURAL PRODUCTS					
BASIC SCREEN			* 95.00*	100,845	
PRIMARY SCREENING, IN VITRO			90.00	95,538	
PRIMARY SCREEN., RELATED NEW MODEL DEVEL			5.00	5,308	
SPARTAN RESEARCH ANIMALS, INC.		N01CM50582 A.P.			109,200
ACQUISITION OF MATERIALS			* 20.00*	21,840	
ANIMALS					
BASIC SCREEN			* 55.00*	60,060	
ANIMALS					
VERIFICATION SCREEN			* 25.00*	27,300	
ANIMALS					
STANFORD RESEARCH INSTITUTE		N01CM33723 D.T.P.			363,474
PROCUREMENT OF PRECLINICAL MATERIAL			* 20.00*	72,695	
ANALYTICAL AND QUALITY CONTROL					
PROD. AND FORM. FOR CLINICAL TRIALS			* 80.00*	290,779	
ANALYTICAL AND QUALITY CONTROL					
STANFORD RESEARCH INSTITUTE		N01CM33742 D.T.P.			508,913
ACQUISITION OF MATERIALS			* 75.00*	381,685	
NEW AGENT PROCUREMENT, SYNTHETICS					
BASIC SCREEN			* 4.00*	20,357	
PRIMARY SCREENING, IN VITRO					
PROCUREMENT OF PRECLINICAL MATERIAL			* 21.00*	106,872	
SYNTHETICS					
STANFORD RESEARCH INSTITUTE		N01CM63776 D.T.P.			239,100
PROCUREMENT OF PRECLINICAL MATERIAL			* 80.00*	191,280	
RADIOLABELED MATERIALS					
PROD. AND FORM. FOR CLINICAL TRIALS			* 20.00*	47,820	
RADIOLABEL					
STANFORD UNIVERSITY		N01CP65825 CTEP			44,100
CLINICAL TRIALS SUPPORTIVE RESEARCH			*100.00*	44,100	
HEAD AND NECK	NUTRITION		20.00	8,820	
LUNG	NUTRITION		20.00	8,820	
OVARIAN	NUTRITION		20.00	8,820	
PANCREATIC	NUTRITION		20.00	8,820	
STOMACH	NUTRITION		20.00	8,820	

NAME	# & AREA	PERCENT OF EFFORT		DOLLAR LEVEL
STANFORD UNIVERSITY, BOARD OF TRUSTEES	N01CM67072 C.O.P.			90,000
CLINICAL TRIALS SUPPORTIVE RESEARCH		*100.00*	90,000	
LYMPHOMA-HISTIOCYT PATHOLOGIC CLASSIF		50.00	45,000	
LYMPHOMA-LYMPHOCYT PATHOLOGIC CLASSIF		50.00	45,000	
STANFORD UNIVERSITY, BOARD OF TRUSTEES	N01CM67111 C.O.P.			84,000
CLINICAL TRIALS SUPPORTIVE RESEARCH		*100.00*	84,000	
LYMPHOMA-HISTIOCYT PATHOLOGIC CLASSIF		50.00	42,000	
LYMPHOMA-LYMPHOCYT PATHOLOGIC CLASSIF		50.00	42,000	
STARKS ASSOCIATES, INC.	N01CM53769 D.T.P.			174,431
PROCUREMENT OF PRECLINICAL MATERIAL SYNTHETICS		* 75.00*	130,823	
PROD. AND FORM. FOR CLINICAL TRIALS PRODUCTION, SYNTHETICS		* 25.00*	43,608	
STARKS ASSOCIATES, INC.	N01CM23203 D.T.P.			279,463
PROCUREMENT OF PRECLINICAL MATERIAL SYNTHETICS		* 50.00*	139,732	
PROD. AND FORM. FOR CLINICAL TRIALS PRODUCTION, SYNTHETICS		* 50.00*	139,732	
STARKS ASSOCIATES, INC.	N01CM53745 D.T.P.			410,404
ACQUISITION OF MATERIALS		*100.00*	410,404	
NEW AGENT PROCUREMENT, SYNTHETICS		70.00	287,283	
DATA PROCESSING AND SUPPORT		30.00	123,121	
STEHLIN FOUNDATION FOR CANCER RESEARCH	N01CM67073 D.T.P.			312,855
BASIC SCREEN PRIMARY SCREEN., RELATED NEW MODEL DEVEL		*100.00*	312,855	
TACONIC FARMS	N01CM50597 A.P.			176,800
ACQUISITION OF MATERIALS ANIMALS		* 20.00*	35,360	
BASIC SCREEN ANIMALS		* 55.00*	97,240	
VERIFICATION SCREEN ANIMALS		* 25.00*	44,200	
TENNESSEE, UNIVERSITY OF	N01CM57021 BCRC			99,797
PROGRAM MANAGEMENT ADMINISTRATION		* 10.00*	9,980	

NAME		# & AREA	PERCENT OF EFFORT		DOLLAR LEVEL
PHASE II CLINICAL TRIALS			* 30.00*	29,939	
CNS TUMORS	CHEM.				
PHASE III CLINICAL TRIALS			* 60.00*	59,878	
CNS TUMORS	RAD.		30.00	29,939	
CNS TUMORS	CHEM.		30.00	29,939	
TEXAS, UNIVERSITY OF, M.D. ANDERSON		N01CM33710 CTEP			184,931
PHASE III CLINICAL TRIALS			*100.00*	184,931	
OVARIAN	CHEM.				
TEXAS, UNIVERSITY OF, M.D. ANDERSON		N01CM77149 CTEP			140,148
PHASE II CLINICAL TRIALS			* 20.00*	28,030	
LUNG	RAD.		10.00	14,015	
LUNG	CHEM.		10.00	14,015	
PHASE III CLINICAL TRIALS			* 60.00*	84,089	
LUNG	RAD.		30.00	42,044	
LUNG	CHEM.		30.00	42,044	
PHASE IV CLINICAL TRIALS			* 20.00*	28,030	
LUNG	RAD.		10.00	14,015	
LUNG	CHEM.		10.00	14,015	
TEXAS, UNIVERSITY OF, M.D. ANDERSON		N01CM53773 D.T.P.			563,605
PHARMACOLOGY/TOXICOLOGY			* 60.00*	338,163	
PHARMACOLOGY					
PHASE I CLINICAL TRIALS			* 40.00*	225,442	
NON-SPECIFIC	PHARM./TOX.				
TEXAS, UNIVERSITY OF, SYSTEM CANCER		N01CM57042 CTEP			618,923
PHASE II CLINICAL TRIALS			* 55.00*	340,408	
BREAST	CHEM.		15.00	92,838	
COLORECTAL	CHEM.		15.00	92,838	
HEAD AND NECK	CHEM.		5.00	30,946	
LUNG	CHEM.		5.00	30,946	
MELANOMA	CHEM.		10.00	61,892	
SARCOMAS (GEN.)	CHEM.		5.00	30,946	
PHASE III CLINICAL TRIALS			* 45.00*	278,515	
BREAST	CHEM.		10.00	61,892	
COLORECTAL	CHEM.		5.00	30,946	
HEAD AND NECK	CHEM.		5.00	30,946	
LUNG	CHEM.		5.00	30,946	
MELANOMA	CHEM.		15.00	92,838	
SARCOMAS (GEN.)	CHEM.		5.00	30,946	
TEXAS, UNIVERSITY OF, SYSTEM CANCER		N01CM67116 CTEP			85,767
PHASE III CLINICAL TRIALS			* 50.00*	42,884	
OVARIAN	SURG.		15.00	12,865	

NAME		# & AREA	PERCENT OF EFFORT		DOLLAR LEVEL
OVARIAN	RAD.		15.00	12,865	
OVARIAN	CHEM.		20.00	17,153	
PHASE IV CLINICAL TRIALS			* 50.00*	42,884	
OVARIAN	SURG.		15.00	12,865	
OVARIAN	RAD.		15.00	12,865	
OVARIAN	CHEM.		20.00	17,153	
TEXAS, UNIVERSITY OF, SYSTEM CANCER		N01CM77153 CTEP			122,968
PHASE II CLINICAL TRIALS			* 20.00*	24,594	
LUNG	RAD.		10.00	12,297	
LUNG	CHEM.		10.00	12,297	
PHASE III CLINICAL TRIALS			* 60.00*	73,781	
LUNG	RAD.		30.00	36,890	
LUNG	CHEM.		30.00	36,890	
PHASE IV CLINICAL TRIALS			* 20.00*	24,594	
LUNG	RAD.		10.00	12,297	
LUNG	CHEM.		10.00	12,297	
TEXAS, UNIVERSITY OF, SYSTEM CANCER		N01CP65794 CTEP			35,096
CLINICAL TRIALS SUPPORTIVE RESEARCH			*100.00*	35,096	
HEAD AND NECK	NUTRITION		20.00	7,019	
LUNG	NUTRITION		20.00	7,019	
OVARIAN	NUTRITION		20.00	7,019	
PANCREATIC	NUTRITION		20.00	7,019	
STOMACH	NUTRITION		20.00	7,019	
TEXAS, UNIVERSITY OF, SYSTEM CANCER		N01CM43801 CTEP			166,672
PHASE I CLINICAL TRIALS			*100.00*	166,672	
BREAST	CHEM.		10.00	16,667	
COLORECTAL	CHEM.		10.00	16,667	
HEAD AND NECK	CHEM.		10.00	16,667	
KIDNEY	CHEM.		10.00	16,667	
LUNG	CHEM.		10.00	16,667	
MELANOMA	CHEM.		15.00	25,001	
PANCREATIC	CHEM.		10.00	16,667	
SARCOMAS (GEN.)	CHEM.		10.00	16,667	
STOMACH	CHEM.		10.00	16,667	
NON-SPECIFIC	CHEM.		5.00	8,334	
TEXAS, UNIVERSITY OF, SYSTEM CANCER		N01CM67026 CTEP			52,603
PHASE III CLINICAL TRIALS			* 50.00*	26,302	
OVARIAN	SURG.		15.00	7,890	
OVARIAN	RAD.		15.00	7,890	
OVARIAN	CHEM.		20.00	10,521	
PHASE IV CLINICAL TRIALS			* 50.00*	26,302	
OVARIAN	SURG.		15.00	7,890	
OVARIAN	RAD.		15.00	7,890	

NAME		# & AREA	PERCENT OF EFFORT		DOLLAR LEVEL
OVARIAN	CHEM.		20.00	10,521	
TEXAS, UNIVERSITY OF, SYSTEM CANCER		N01CM43786 D.T.P.			64,170
VERIFICATION SCREEN			*100.00*	64,170	
DETAILED DRUG EVALUATION, IN VITRO			60.00	38,502	
DETAILED DRUG EVALUATION, IN VIVO			20.00	12,834	
DET. DRUG EVAL., RELATED NEW MODEL DEVEL			20.00	12,834	
TEXAS, UNIVERSITY OF		N01CM53767 D.T.P.			84,357
VERIFICATION SCREEN			*100.00*	84,357	
DET. DRUG EVAL., RELATED NEW MODEL DEVEL					
TEXAS, UNIVERSITY OF		N01CM53832 C.O.P.			628,793
CLINICAL TRIALS SUPPORTIVE RESEARCH			*100.00*	628,793	
LEUK.-ACUTE GRAN.	PROTECTED ENVIRON.		20.00	125,759	
LEUK.-ACUTE LYMPH.	PROTECTED ENVIRON.		40.00	251,517	
SARCOMAS (GEN.)	PROTECTED ENVIRON.		40.00	251,517	
UPJOHN COMPANY		N01CM77100 D.T.P.			787,152
ACQUISITION OF MATERIALS			* 85.00*	669,079	
NEW AGENT PROCUREMENT, FERMENT/ANTIBIOT			83.00	653,336	
DATA PROCESSING AND SUPPORT			2.00	15,743	
BASIC SCREEN			* 15.00*	118,073	
PRIMARY SCREENING, IN VITRO			10.00	78,715	
PRIMARY SCREEN., RELATED NEW MODEL DEVEL			5.00	39,358	
UPJOHN COMPANY		N01CM43753 D.T.P.			440,225
ACQUISITION OF MATERIALS			* 57.00*	250,928	
NEW AGENT PROCUREMENT, FERMENT/ANTIBIOT			55.00	242,124	
DATA PROCESSING AND SUPPORT			2.00	8,805	
BASIC SCREEN			* 17.00*	74,838	
PRIMARY SCREENING, IN VITRO			7.00	30,816	
PRIMARY SCREENING, IN VIVO			3.00	13,207	
PRIMARY SCREEN., RELATED NEW MODEL DEVEL			7.00	30,816	
FORMULATION			* 1.00*	4,402	
DEVEL. OF EXP. FORMULATIONS					
VERIFICATION SCREEN			* 10.00*	44,023	
MOLECULAR BIOLOGY					
PHARMACOLOGY/TOXICOLOGY			* 15.00*	66,034	
PHARMACOLOGY			13.00	57,229	
DATA PROCESSING AND SUPPORT			2.00	8,805	
VALUE ENGINEERING COMPANY		N01CM53821 D.T.P.			125,000
PROGRAM MANAGEMENT			*100.00*	125,000	
DATA PROCESSING AND SUPPORT					

NAME		# & AREA	PERCENT OF EFFORT		DOLLAR LEVEL
VALUE ENGINEERING COMPANY		N01CM67086 D.T.P.			936,242
ACQUISITION OF MATERIALS			* 14.00*	131,074	
BIOASSAY OF NATURAL PRODUCTS					
BASIC SCREEN			* 58.00*	543,020	
PRIMARY SCREENING, IN VITRO			5.00	46,812	
PRIMARY SCREENING, IN VIVO			53.00	496,208	
VERIFICATION SCREEN			* 20.00*	187,248	
DETAILED DRUG EVALUATION, IN VIVO					
TREATMENT STUDIES			* 8.00*	74,899	
CHEMOTHERAPY					
VANDERBILT UNIVERSITY MEDICAL		N01CM77122 CTEP			108,433
PHASE II CLINICAL TRIALS			* 20.00*	21,687	
LUNG	RAD.		10.00	10,843	
LUNG	CHEM.		10.00	10,843	
PHASE III CLINICAL TRIALS			* 60.00*	65,060	
LUNG	RAD.		30.00	32,530	
LUNG	CHEM.		30.00	32,530	
PHASE IV CLINICAL TRIALS			* 20.00*	21,687	
LUNG	RAD.		10.00	10,843	
LUNG	CHEM.		10.00	10,843	
VERMONT, UNIVERSITY OF, COLLEGE OF		N01CM33716 BCRC			40,000
PROGRAM MANAGEMENT			* 20.00*	8,000	
ADMINISTRATION			1.00	400	
COMMUNICATION AND EDUCATION			9.00	3,600	
DATA PROCESSING AND SUPPORT			10.00	4,000	
PHASE III CLINICAL TRIALS			* 80.00*	32,000	
LEUK.-ACUTE GRAN.	SUPPORTIVE CARE		50.00	20,000	
NON-SPECIFIC	CHEM.		30.00	12,000	
VETERANS ADMINISTRATION		Y01CM70106 C.O.P.			2,157,000
PHASE III CLINICAL TRIALS			* 40.00*	862,800	
HEAD AND NECK	CHEM.		5.00	107,850	
HEAD AND NECK	IMM.		5.00	107,850	
LUNG	RAD.		10.00	215,700	
LUNG	CHEM.		20.00	431,400	
PHASE IV CLINICAL TRIALS			* 40.00*	862,800	
COLORECTAL	SURG.		5.00	107,850	
COLORECTAL	RAD.		5.00	107,850	
COLORECTAL	CHEM.		5.00	107,850	
ESOPHAGEAL	RAD.		2.50	53,925	
ESOPHAGEAL	CHEM.		2.50	53,925	
LUNG	SURG.		5.00	107,850	
LUNG	CHEM.		5.00	107,850	
LUNG	IMM.		5.00	107,850	

NAME		# & AREA	PERCENT OF EFFORT		DOLLAR LEVEL
PANCREATIC	CHEM.		2.50	53,925	
STOMACH	CHEM.		2.50	53,925	
OTHER CLINICAL TRIALS RESEARCH			* 20.00*	431,400	
COLORECTAL	DATA PROC. & SUPP.		2.50	53,925	
ESOPHAGEAL	DATA PROC. & SUPP.		2.50	53,925	
HEAD AND NECK	DATA PROC. & SUPP.		2.50	53,925	
LUNG	DATA PROC. & SUPP.		5.00	107,850	
PANCREATIC	DATA PROC. & SUPP.		2.50	53,925	
STOMACH	DATA PROC. & SUPP.		5.00	107,850	
VETERANS ADMINISTRATION		Y01CM70107 CTEP			1,376,000
PHASE III CLINICAL TRIALS			* 40.00*	550,400	
HEAD AND NECK	CHEM.		5.00	68,800	
HEAD AND NECK	IMM.		5.00	68,800	
LUNG	RAD.		10.00	137,600	
LUNG	CHEM.		20.00	275,200	
PHASE IV CLINICAL TRIALS			* 40.00*	550,400	
COLORECTAL	SURG.		5.00	68,800	
COLORECTAL	RAD.		5.00	68,800	
COLORECTAL	CHEM.		5.00	68,800	
ESOPHAGEAL	RAD.		2.50	34,400	
ESOPHAGEAL	CHEM.		2.50	34,400	
LUNG	SURG.		5.00	68,800	
LUNG	CHEM.		5.00	68,800	
LUNG	IMM.		5.00	68,800	
PANCREATIC	CHEM.		2.50	34,400	
STOMACH	CHEM.		2.50	34,400	
OTHER CLINICAL TRIALS RESEARCH			* 20.00*	275,200	
COLORECTAL	DATA PROC. & SUPP.		2.50	34,400	
ESOPHAGEAL	DATA PROC. & SUPP.		2.50	34,400	
HEAD AND NECK	DATA PROC. & SUPP.		2.50	34,400	
LUNG	DATA PROC. & SUPP.		5.00	68,800	
PANCREATIC	DATA PROC. & SUPP.		2.50	34,400	
STOMACH	DATA PROC. & SUPP.		5.00	68,800	
VETERANS ADMINISTRATION		Y01CM60103 D.T.P.			554
PHARMACOLOGY/TOXICOLOGY SPECIAL TOXICITY STUDIES			*100.00*	554	
VETERANS ADMINISTRATION		Y01CM60102 D.T.P.			50,104
ACQUISITION OF MATERIALS NEW AGENT PROCUREMENT, SYNTHETICS			* 90.00*	45,094	
PROCUREMENT OF PRECLINICAL MATERIAL SYNTHETICS			* 10.00*	5,010	
VIRGINIA, UNIVERSITY OF		N01CM57002 D.T.P.			157,363
ACQUISITION OF MATERIALS			* 85.00*	134,269	

NAME	# & AREA	PERCENT OF EFFORT		DOLLAR LEVEL
NEW AGENT PROCUREMENT, PLANT PRODUCTS		65.00	102,676	
NEW AGENT PROCUREMENT, FERMENT/ANTIBIOT		15.00	23,694	
BIOASSAY OF NATURAL PRODUCTS		5.00	7,898	
PROCUREMENT OF PRECLINICAL MATERIAL		* 15.00*	23,694	
PLANT PRODUCTS		10.00	15,796	
FERMENTATION/ANTIBIOTICS		5.00	7,898	
WALTER REED ARMY MEDICAL CENTER	Y01CM40005 CTEP			62,099
BIOLOGICAL STUDIES		*100.00*	62,099	
DIAGNOSIS				
WARF INSTITUTE, INC.	N01CM43707 D.T.P.			120,666
ACQUISITION OF MATERIALS		*100.00*	120,666	
NEW AGENT PROCUREMENT, PLANT PRODUCTS				
WARF INSTITUTE, INC.	N01CM53770 D.T.P.			696,922
ACQUISITION OF MATERIALS		* 50.00*	348,461	
BIOASSAY OF NATURAL PRODUCTS				
BASIC SCREEN		* 40.00*	278,769	
PRIMARY SCREENING, IN VIVO		36.00	250,892	
PRIMARY SCREEN., RELATED NEW MODEL DEVEL		4.00	27,877	
VERIFICATION SCREEN		* 9.00*	62,723	
DETAILED DRUG EVALUATION, IN VIVO				
TREATMENT STUDIES		* 1.00*	6,969	
CHEMOTHERAPY				
WASHINGTON UNIVERSITY	N01HB62970 O.D.			121,511
CLINICAL TRIALS SUPPORTIVE RESEARCH		*100.00*	121,511	
OTHER TRANSFUSION RES.				
WASHINGTON UNIVERSITY SCHOOL OF	N01CM43731 D.T.P.			84,182
VERIFICATION SCREEN		* 50.00*	42,091	
DETAILED DRUG EVALUATION, IN VIVO				
TREATMENT STUDIES		* 50.00*	42,091	
COMB. MODAL. THERAPY		50.00	42,091	
COMB. MODAL. THERAPY - CHEM.				
WAYNE STATE UNIVERSITY	N01CM67105 CTEP			444,666
PHASE II CLINICAL TRIALS		* 55.00*	244,566	
BREAST CHEM.		15.00	66,700	
COLORECTAL CHEM.		15.00	66,700	
HEAD AND NECK CHEM.		5.00	22,233	
LUNG CHEM.		5.00	22,233	
MELANOMA CHEM.		10.00	44,467	
SARCOMAS (GEN.) CHEM.		5.00	22,233	

NAME		# & AREA	PERCENT OF EFFORT		DOLLAR LEVEL
PHASE III CLINICAL TRIALS			* 45.00*	200,100	
BREAST	CHEM.		10.00	44,467	
COLORECTAL	CHEM.		5.00	22,233	
HEAD AND NECK	CHEM.		5.00	22,233	
LUNG	CHEM.		5.00	22,233	
MELANOMA	CHEM.		15.00	66,700	
SARCOMAS (GEN.)	CHEM.		5.00	22,233	
WISCONSIN, UNIVERSITY OF		N01CM67076 D.T.P.			59,624
ACQUISITION OF MATERIALS			* 5.00*	2,981	
BIOASSAY OF NATURAL PRODUCTS					
BASIC SCREEN			* 95.00*	56,643	
PRIMARY SCREENING, IN VITRO			90.00	53,662	
PRIMARY SCREEN., RELATED NEW MODEL DEVEL			5.00	2,981	
YALE UNIVERSITY SCHOOL OF MEDICINE		N01CM43797 CTEP			131,179
PHASE II CLINICAL TRIALS			* 30.00*	39,354	
PANCREATIC	CHEM.				
PHASE IV CLINICAL TRIALS			* 50.00*	65,590	
PANCREATIC	SURG.		5.00	6,559	
PANCREATIC	RAD.		20.00	26,236	
PANCREATIC	CHEM.		25.00	32,795	
OTHER CLINICAL TRIALS RESEARCH			* 20.00*	26,236	
PANCREATIC	DATA PROC. & SUPP.				
YALE UNIVERSITY SCHOOL OF MEDICINE		N01CM53843 CTEP			96,449
PHASE IV CLINICAL TRIALS			* 90.00*	86,804	
STOMACH	SURG.		25.00	24,112	
STOMACH	RAD.		25.00	24,112	
STOMACH	CHEM.		40.00	38,580	
CLINICAL TRIALS SUPPORTIVE RESEARCH			* 10.00*	9,645	
STOMACH	MARKERS				
YALE UNIVERSITY SCHOOL OF MEDICINE		N01CM67068 D.T.P.			50,000
BASIC SCREEN			* 30.00*	15,000	
PRIMARY SCREENING, IN VITRO					
PHARMACOLOGY/TOXICOLOGY			* 35.00*	17,500	
PHARMACOLOGY			20.00	10,000	
OTHER			15.00	7,500	
PHASE I CLINICAL TRIALS			* 35.00*	17,500	
NON-SPECIFIC	PHARM./TOX.				
YALE UNIVERSITY SCHOOL OF MEDICINE		N01CM53824 D.T.P.			69,856
ACQUISITION OF MATERIALS			* 67.00*	46,804	
NEW AGENT PROCUREMENT, SYNTHETICS					
BASIC SCREEN			* 33.00*	23,052	

NAME	# & AREA	PERCENT OF EFFORT		DOLLAR LEVEL
PRIMARY SCREENING, IN VITRO		30.00	20,957	
PRIMARY SCREENING, IN VIVO		3.00	2,096	
YALE UNIVERSITY SCHOOL OF MEDICINE	N01CM57029 D.T.P.			99,110
ACQUISITION OF MATERIALS		* 75.00*	74,333	
NEW AGENT PROCUREMENT, SYNTHETICS				
BASIC SCREEN		* 25.00*	24,778	
PRIMARY SCREENING, IN VITRO		12.00	11,893	
PRIMARY SCREENING, IN VIVO		13.00	12,884	

TABLE VII
DESCRIPTION OF CONTRACTS IN THE DIVISION OF CANCER TREATMENT PROGRAM

ABBOTT LABORATORIES (N01-CM4-3704)

This contract was designed to develop a conditioning medium that will cause normal and leukemic granulocytic precursor cells to differentiate and form colonies of mature granulocytes. This "colony stimulating factor" has now been isolated, purified and characterized. The terminal year of this contract will be spent preparing enough material for a clinical trial in 50 patients. An IND is being submitted.

AGRICULTURE, DEPARTMENT OF (TRANSFER OF FUNDS) (Y01-CM4-0001)

Under the Transfer of Funds Agreement with the Medicinal Plant Resources Laboratory of the U.S.D.A., worldwide general plant collections and recollections are made. The general plant collections (approximately 1000 plants) are shipped to WARF Institute, Inc. for extraction and the extracts are screened *in vitro* and *in vivo* for antineoplastic activity. In the last year 183 plant extracts have confirmed *in vivo* PS activity and 124 plant extracts have confirmed *in vitro* KB cell cytotoxicity. Confirmed active plants are recollected in large quantities (300-500 lbs.) on a priority based on the needs of the Division of Cancer Treatment Program for shipment to the fractionating chemists. Very large recollections are made, as requested by the Developmental Therapeutics Program, for the preparation of clinical quantities of drugs. The nomenclature of all plants acquisitioned is being continuously reviewed and corrected, and a computer file of all plants is being maintained.

ALABAMA, UNIVERSITY OF (N01-CM6-7083)

This contractor operates primarily at the genetic center level, making certain that isolator reared progeny from the caesarian-derived animals contain no undesirable organisms. Contractor also coordinates virus diagnostic results with his own observations of pathological lesions in arriving at a definitive diagnosis when disease problems occur.

ALBANY MEDICAL COLLEGE (N01-CM5-7032)

This contract is designed to support prospective, randomized controlled protocol studies using combined modalities in the surgical adjuvant treatment of colon and rectal carcinoma.

ALBERT EINSTEIN COLLEGE OF MEDICINE (N01-CM4-3787) (FORMERLY YESHIVA UNIVERSITY)

The major emphasis of this project is to provide a detailed evaluation of new antitumor drugs which affect protein synthesis. For drugs having significant effects on protein synthesis, detailed studies on messenger binding, initiation, translocation and peptide bond formation will be done.
In addition, detailed studies on the mechanism of action of new antitumor drugs which may directly affect the structure of DNA are performed.

ALDRICH CHEMICAL COMPANY, INC. (N01-CM2-3706)

This service preparative contract provides for the resynthesis of a variety of compounds required for clinical or preclinical evaluation. The compounds prepared are not readily available on the open market or from the original supplier in the amounts required. About 30% of the effort of this contract is devoted to the preparation of large quantities of materials, in the multi-kilogram range, requiring pilot plant facilities.

ALLEGHENY GENERAL HOSPITAL (N01-CM4-3730)

The objective of this contract is to determine the dose and time course effects of antitumor drugs selected by the National Cancer Institute on survival of normal gastrointestinal crypts and on the *in vivo* survival of experimental murine mastocytoma cells. The effects of multiple drug dose interval on the normal gastrointestinal mucosa and the mastocytoma (in various metastatic sites) will be evaluated. Information on dose schedules and drug combinations leading to minimal or maximal gastrointestinal toxicity will be provided concurrent with the effects of these therapies on a proliferating murine mastocytoma. The methods used in these studies to determine gastrointestinal crypt survival were developed by the Contractor. This contract terminated December 1976.

ARIZONA, UNIVERSITY OF (N01-CM3-3713)

This contract is devoted to the further development of a staging system for patients with multiple myeloma. The rationale for this proposed staging system was based on detailed studies by the principle investigator, Dr. Sydney Salmon, in working out a procedure for determining total myeloma cell number in patients. The proposed system incorporates available clinical and laboratory data and measurements in conjunction with tumor cell number to derive a means for staging myeloma patients. This system is now being tested prospectively to determine if the desired correlations with actual tumor cell measurements hold true. It is anticipated this system could be used for evaluation of the number of tumor cells remaining following therapy using clinical parameters without the necessity for carrying out the detailed and involved procedures for actual measurement of this number. This contract will terminate June 1977.

ARIZONA BOARD OF REGENTS (UNIVERSITY OF ARIZONA) (N01-CM3-3750)

The major objective of this contract is the fractionation of confirmed active plants in order to isolate in a pure state and identify the antitumor active components. Recent promising results have been obtained from the extraction of plants collected in the Western Hemisphere. Further fractionation is being carried out to isolate the active components. A KB cell culture unit has been added to expedite the fractionation work. This contract will terminate June 1977.

ARIZONA STATE UNIVERSITY (N01-CM6-7048) (FORMERLY ARIZONA BOARD OF REGENTS N01-CM1-2308)

The major objective of this contract is the preparation, from animal materials of marine species, of extracts suitable for bioassay in the National Cancer Institute antitumor screen. The extracts are prepared in accordance with procedures acceptable to the National Cancer Institute. Extracts showing preliminary activity are prepared anew for confirmatory testing and materials showing confirmed activity are assigned for fractionation and isolation.

ARMY RESEARCH INSTITUTE OF ENVIRONMENTAL MEDICINE (Y01-CM6-0105)

Apparatus for the production and control of whole body hyperthermia will be studied and evaluated with regard to human response to heat at extreme body temperatures. Physiological, biochemical, psychological, pathological and clinical responses of human subjects exposed to experimental heat stress will be performed. The data obtained will be integrated, with thermal manikin data, into a predictive model. Model verification and perturbation will be undertaken in collaboration with NCI personnel at the NIH Clinical Center. Support personnel will be provided to assist and instruct NCI researchers in the use and operation of whole body hyperthermia apparatus at the NIH Clinical Center. Such additional collaborative hyperthermia studies as are of mutual interest will be undertaken.

ARS/SPRAGUE-DAWLEY (N01-CM5-3813)

This primary genetic center produces a variety of outbred, inbred and hybrids of inbred rodents. All production activities are effected in a closely controlled environment.

ARS/SPRAGUE-DAWLEY (N01-CM5-3846)

This contract provides for the maintenance of a rodent production center. This center produces stocks and strains of rodents for research and testing laboratory programs. Production levels for individual colonies are correlated with requirements for specific investigations. This contract also furnishes breeding animals for large-scale production colonies. Major emphasis is upon the production of C57BL/6, C3H/He MTV+, DBA/8, Balb/c-CMC, and CD8F1 mice.

ARTHUR D. LITTLE, INC. (N01-CM3-3727)

This applied mathematics and data analysis resource project supplies biostatistical support to the Drug Evaluation Branch, Developmental Therapeutics Program, Division of Cancer Treatment, specifically to aid in establishing parameters and criteria of activity and toxicity in current and new antitumor screens, in formulating detailed experimental protocols for use in contract testing laboratories, and in maintaining testing quality control by evaluating screening methodology with respect to test-to-test and lab-to-lab variability. The workscope includes studies of individual screening models (animal tumor assays) with regard to their validity (e.g., reproducibility) and their yield of active materials and to determine the degree of correlations among alternative models. Additionally, this project includes tasks related to the evaluation and summarization of clinical results. Analysis of data from antitumor screening laboratories and from the clinic as well provides for the quantitative assessment of correlations between activity against specific laboratory models and activity against specific human malignancies.

ARTHUR D. LITTLE, INC. (N01-CM5-3765)

The three principal facets of this project are: (1) in vivo antitumor screening of structural congeners, "analogs", of known antitumor drugs to aid in assessing the potential superiority of an analog over the known parent compound and to supply results of testing directly and rapidly to organic chemists to aid the direction of new agent synthesis - the test systems used are designed specifically for each compound class of interest; (2) studies of combinations of drugs to determine their potential superiority over the individual drugs; and (3) studies of the effects of radiation therapy and combinations of drugs plus radiation and/or surgery. Considerable emphasis is placed on the quantitative comparative evaluation in vivo of potential radiation-sensitizing chemical agents.

ARTHUR D. LITTLE, INC. (N01-CM5-3849)

This contract encompasses three distinct work areas: (1) physiological disposition and biochemical pharmacology, (2) electron microscopy and radioautography, and (3) tumor bank distribution services. Under the first activity, work is conducted on the physiological disposition and biochemical pharmacology of antitumor agents under active development in the Division of Cancer Treatment Program. Work has been conducted on PALA, Ftorafur, Ellipticine, Tritylcysteine, 5-azacytidine plus THU and Selenoguanosine. Electron microscopy and radioautography have focused on the whole body radioautography of Adriamycin and Daunomycin, 5-FU and Ftorafur, 2,4, and 6 C^{14} 5-azacytidine, electron microscopy of the Nettesheim Tumor and the effect of Vitamin E on the Adriamycin-induced cardiomyopathy in mouse heart. Finally, approximately 400 tumor sublines have been shipped to screening laboratories and investigators around the globe and a bank of almost 750 tumor sublines are maintained continuously.

ARTHUR D. LITTLE, INC. (N01-CM5-7006)

This is a service contract for the *in vivo* screening of new synthetic and natural product materials and the follow-up testing of active materials. The primary screening is carried out in accordance with Developmental Therapeutics Program protocols. New materials are tested against the P388 leukemia test system. As requested, testing is also carried out in the panel of antitumor test systems. Detailed testing, such as schedule dependency studies in the active tumor system, is conducted with materials of potential clinical interest. Methodology studies, such as the characterization and evaluation of tumor test systems, are carried out as directed by the Project Officer. The current level of testing is approximately 25,000 L1210 equivalent tests per year.

ARTHUR D. LITTLE, INC. (N01-CM6-3823)

This contract provides broad biomedical engineering resources which respond to individual research projects within the Division of Cancer Treatment. A flexible mechanism of support defines and implements exploratory engineering projects in close collaboration with the DCT staff in diverse areas covering instrumentation and mathematical modeling for tumor cell kinetics, automated microspectrometry, novel clinical equipment development, and thermometry for hyperthermia studies, etc. A "core group" of senior engineers continually consults and interacts with the DCT staff on the definition of problems and projects while "project groups" are tailored to implement specific engineering tasks under NCI staff direction.

ARTHUR D. LITTLE, INC. (N01-CM6-7047)

The workscope of this program is to extend the research objectives of the Central Nervous System Solid Tumor Studies with the neonate-puppy animal model. This will include the transplantation of gliomas in the CNS of neonate beagle puppies to provide suitable tumor materials for chemotherapy studies; chemotherapy studies with selected agents against the transplantable gliosarcoma in the brains of newborn puppies; histopathologic evaluation of the effects of chemotherapeutic agents against the transplantable gliosarcoma in the brains of newborn puppies; collaborative studies with clinical and pharmacology groups of the National Cancer Institute to study the growth kinetics and biologic behavior of transplantable gliomas to selected agents; methodological studies as agreed upon with the approval of the Project Officer and exploring the use of the transplantable beagle tumor in heterologous hosts such as rodents (rats, hamsters).

ARTHUR D. LITTLE, INC. (N01-CM6-7078)

This is a service contract for *in vitro* testing of crude natural products, fractions of confirmed natural products, continuation of analogue evaluation (see contract N01-CM5-3765), and synthetic materials - new to the program - submitted in too small quantity to permit *in vivo* testing. The 9KB and P388 leukemias are routinely used in the performance of this contract. Due to the lack of materials to be tested in the L1210 system, this tumor has been frozen and will be reactivated, if the need should arise. Testing is being conducted at the level of 6,000 9KB equivalents per year. Research and development is conducted at the request of the Drug Evaluation Branch.

ASH STEVENS, INC. (N01-CM5-7012)

This service preparative contract provides for the resynthesis of a variety of compounds required for clinical or preclinical evaluation. The compounds prepared are not readily available from the original supplier or commercial sources in the amounts required. About 75% of the effort of this contract is devoted to the preparation of large quantities of materials, in the multikilogram range, requiring pilot plant facilities.

ASSOCIATED BIOMEDIC SYSTEMS, INC. (N01-CM0-2271)

The purposes of this contract are: (1) to supply normal human blood lymphocytes, both untreated and stimulated with phytohemagglutinin; (2) to produce and evaluate human myeloid colony-stimulating factor(s) by "conditioning" media with various cells and then testing such media for "factor" activity; (3) to perform various services regarding culture of human cell lines such as (a) mass production of "interesting" lines (e.g., cells containing RNA-directed DNA polymerase, DNA polymerase γ, terminal deoxynucleotidyl transferase, or cell producing "factors"), and (b) ridding cell lines of contaminating mycoplasma by various techniques. Certain cell-free "conditioned" media have been identified as rich sources of colony-stimulating factor.

BATTELLE MEMORIAL INSTITUTE (N01-CM4-3746)

In March, 1974, a prime contract was awarded with Battelle Memorial Laboratories for supervision of all contracts carrying out the toxicologic evaluation of potential oncolytic agents under the aegis of the Laboratory of Toxicology. Through the prime contract mechanism, preclinical toxicologic studies of agents under consideration for potential clinical use are handled under a single management-type contract. The workscope is comprised of four tasks as follows: Task I - Full Protocol Studies; Task II - High Priority Toxicity Studies (i.e., any portion of the Protocol of the Laboratory of Toxicology); Task III - Specific Organ Testing; and Task IV - Automation of toxicity data, anomaly detection, scheduling of studies, and financial management.

BATTELLE MEMORIAL INSTITUTE (N01-CM5-7005)

Battelle Columbus Laboratories conduct *in vivo* testing of new synthetic materials and natural products, of both plant and animal origin, in addition to the secondary evaluation of active materials of interest to the Developmental

BATTELLE MEMORIAL INSTITUTE (N01-CM5-7005) (CONTINUED)

Therapeutics Program. This testing is conducted at a level of effort equivalent to approximately 50,000 leukemia L1210 tests per annum. Testing is conducted according to current Developmental Therapeutics Program protocols, i.e., new synthetic materials and new natural products are tested in the P388 leukemia. When requested, Battelle Columbus schedules the secondary evaluation of materials of program interest in the tumor panel systems. Schedule dependency studies are also conducted with materials of potential clinical interest. Tumors are maintained in a tumor bank and are available to the Developmental Therapeutics Program. The Project Officer, dependent upon the current needs of the Drug Evaluation Branch, may change the tumor assay systems.

BATTELLE MEMORIAL INSTITUTE (N01-CM6-7099)

This contract provides for the development of protocols and the screening of compounds on human tumor xenografts in athymic mice. Currently, three human breast, four colon and one lung tumor are under study for possible selection for use in the tumor panel. Protocols are under development and evaluation criteria are being analyzed.

BAYLOR COLLEGE OF MEDICINE (N01-CM5-7018)

The purpose of this contract is to evaluate the usefulness of the hyperplastic nodule system (HAN) in the selection of potential anticancer agents for the clinic. Hyperplastic nodules are mammary alveolar tissue from female mice which is altered from normal but is distinct from malignant tissue. These nodules grow in cleared mammary fat pads and may be carried indefinitely by transplant. A number of stable lines have been established, from each of which mammary tumors arise over a characteristic time interval and grow in a uniform manner. The HAN system provides reproducible spontaneous mammary tumors for testing a limited number of drugs. The effects on three parameters are possible: (1) on the growth of the hyperplastic nodule transplant in the fat pad, (2) on numbers and time of appearance of tumors, and (3) on tumor growth. The contract is to be phased out June 1977.

BELLAIRE ACRES (N01-CM5-0583) (FORMERLY NIH-75-C-583)

This contract furnishes approximately 312,000 CD2F1 (BALB/c female x DBA/2 male) hybrid mice for compound evaluation studies. Breeding animals are furnished from genetic centers and/or rodent production colonies.

BEN VENUE LABORATORIES, INC. (N01-CM6-7109) (FORMERLY N01-CM5-3811)

This service contract provides for the development and production of parenteral clinical dosage forms of antitumor agents. Ben Venue Laboratories, Inc. has the capacity for preparing large-scale production batches of dry-filled, liquid-filled and lyophilized sterile products. Specifically, the Contractor performs the following services: (1) formulation development of sterile parenteral products, (2) production and quality assurance testing of parenteral dosage forms for clinical use, and (3) stability surveillance of all dosage forms produced. All products produced are packaged, labelled and shipped to the National Cancer Institute for subsequent redistribution to clinical investigators.

BIOTECH RESEARCH LABORATORIES, INC. (N01-CM6-7052)

This contract has the objective of implementing and operating an in vitro primary screen for identification of drugs with potential therapeutic activity in established tumors and in the remission maintenance of treated tumors. Specifically the screen is designed to detect (a) drug activity against cells with oncogenic virus gene-directed phenotypic properties, and (b) drug activity against those oncogenic virus properties associated with tumor induction of reinduction.

BOWMAN GRAY SCHOOL OF MEDICINE (N01-CM6-7054)

The clinical data from brain tumor patients provided under this contract are used to evaluate the efficacy of therapy in both Phase II and Phase III clinical trials. Single agent treatment as well as combined modalities therapy are used as detailed in the protocols of the Brain Tumor Study Group. The Phase III trials evaluate chemotherapy, radiation therapy and combinations thereof (multimodality therapy) following definitive surgery in the treatment of malignant gliomas. New chemotherapeutic agents are also being tested in Phase II trials in order to make the selection of chemotherapeutic agents for future Phase III trials of brain tumor patients more meaningful. The acquisition of patients into these prospective, controlled, randomized studies permits a comparative analysis of the clinical course of the patient and the toxicity of the various therapeutic modalities utilized as well as insight into the biological characteristics of the disease entity being investigated.

BRIGHAM YOUNG UNIVERSITY (N01-CM4-3790)

The major emphasis of this project is detailed evaluation of selected new antitumor drugs on folic acid metabolism. In addition to a study of drug action on thymidylate synthetase and dihydrofolate reductase, drug effects on serine transhydroxymethylase, N^5, N^{10}-methylene THF reductase, N^5-methyl THF homocyteine transmethylase, N^5, N^{10}-methylene THF dehydrogenase, N^5, N^{10}-methenyl THF cyclohydrase, 5-amino, 4-imidazolecarboxamide ribonucleotide transformylase and glycinamide ribonucleotide transformylase will be studied.

BRISTOL-MYERS COMPANY (N01-CM3-3751)

This is a no-cost contract concerned with the marketing of the clinically useful nitrosourea, BCNU. The Contractor summarized the clinical and other data, and prepared and filed the New Drug Application (NDA) with the Food and Drug Administration. The NDA has recently been approved and the contractor will have the drug on the market by May 1977.

BRISTOL-MYERS COMPANY (N01-CM4-3759)

The major objective of this fermentation contract is the preparation of novel antibiotics. These new antibiotics are obtained using the fermentation techniques of biotransformation and co-metabolism of antibiotics, chemicals and plant materials. This effort is to convert materials which have solubility, toxic or marginal activity problems into worthwhile antineoplastic agents. A multitude of unique organisms is isolated and used to obtain these microbial conversions in an effort to obtain new antineoplastic agents.

BRISTOL-MYERS COMPANY (N01-CM6-7051)

This is a no-cost contract concerned with the marketing of the clinically useful nitrosourea, CCNU. The Contractor summarized the clinical and other data, and prepared and filed the New Drug Application (NDA) with the Food and Drug Administration. The NDA has been approved and the drug is currently on the market.

BRISTOL-MYERS COMPANY (N01-CM7-7147)

This contract includes the isolation of unique organisms using unusual techniques, the fermentation of these microbes to produce novel antineoplastic agents, and the production of sufficient material for thorough NCI evaluation. The areas of fermentation co-metabolism are being explored. The design of new pre-screens is also being evaluated.

BRITISH COLUMBIA, UNIVERSITY OF (N01-CM2-3223)

The main objective of this contract is to develop a versatile, yet commercially feasible synthesis of the dimeric vinca alkaloids, vincristine and vinblastine, and structurally related analogs. The "biogenetic approach" that was initiated shows considerable versatility, a patent application has been filed, and it is certain that this approach will not only lead to a commercially feasible route to the vinca alkaloids, but will also provide novel compounds for screening. In fact, a number of synthetic derivatives have shown antitumor activity comparable to that of vinblastine and/or vincristine, but markedly reduced toxicity.

CALIFORNIA, UNIVERSITY OF (LOS ANGELES) (N01-CM0-2092)

This contract was designed to provide HL-A typing analyses on patients and their families as well as typing of selected donors from the Clinical Center Blood Bank and all platelet donors from the Central Blood Service of Baltimore. Typing data on over 15,000 NIH donors and recipients are on computer file. All typings are performed and entered into the computerized file on the same day that the blood was received. Thus, HL-A typing results for persons bled in Bethesda are consistently available through the computer terminal by the end of the next day. An average of 600 typings are performed annually.

CALIFORNIA, UNIVERSITY OF (LOS ANGELES) (N01-CM4-3791)

The goal of this project is to develop and use assays which are capable of detecting and evaluating antitumor agents which can increase the lethal effects of ionizing radiation _in vitro_ and _in vivo_. Such effects will be evaluated by cytocidal activity in cell culture, by effects on the cell's ability to repair x-ray induced DNA damage and by effects on the growth of locally implanted Lewis lung carcinoma _in vivo_.

CALIFORNIA, UNIVERSITY OF (LOS ANGELES) (N01-CM4-3798)

This contract is designed to evaluate a combined modality protocol employing radiotherapy and chemotherapy in the treatment of locally unresectable carcinoma of the pancreas.

CALIFORNIA, UNIVERSITY OF (LOS ANGELES) (N01-CM5-3752)

The clinical data from brain tumor patients provided under this contract are used to evaluate the efficacy of therapy in both Phase II and Phase III clinical trials. Single agent treatment as well as combined modalities therapy are used as detailed in the protocols of the Brain Tumor Study Group. The Phase III trials evaluate chemotherapy, radiation therapy and combinations thereof (multimodality therapy) following definitive surgery in the treatment of malignant gliomas. New chemotherapeutic agents are also being tested in Phase II trials in order to make the selection of chemotherapeutic agents for future Phase III trials of brain tumor patients more meaningful. The acquisition of patients into these prospective, controlled, randomized studies permits a comparative analysis of the clinical course of the patient and the toxicity of the various therapeutic modalities utilized as well as insight into the biological characteristics of the disease entity being investigated.

CALIFORNIA, UNIVERSITY OF (SAN FRANCISCO) (N01-CM6-7055)

The clinical data from brain tumor patients provided under this contract are used to evaluate the efficacy of therapy in both Phase II and Phase III clinical trials. Single agent treatment as well as combined modalities therapy are used as detailed in the protocols of the Brain Tumor Study Group.

CALIFORNIA, UNIVERSITY OF (SAN FRANCISCO) (N01-CM6-7055) (CONTINUED)

The Phase III trials evaluate chemotherapy, radiation therapy and combinations thereof (multimodality therapy) following definitive surgery in the treatment of malignant gliomas. New chemotherapeutic agents are also being tested in Phase II trials in order to make the selection of chemotherapeutic agents for future Phase III trials of brain tumor patients more meaningful. The acquisition of patients into these prospective, controlled, randomized studies permits a comparative analysis of the clinical course of the patient and the toxicity of the various therapeutic modalities utilized as well as insight into the biological characteristics of the disease entity being investigated.

CALIFORNIA, UNIVERSITY OF (LOS ANGELES) (N01-CM6-7097)

This contract is designed to support prospective randomized, controlled protocol studies using combined modalities in the surgical adjuvant treatment of colon and rectal carcinoma.

CALIFORNIA, UNIVERSITY OF (LOS ANGELES) (N01-CM7-7151)

A member of the Lung Cancer Study Group: A collaborative group of six (6) institutions which are studying early non-oat cell lung cancer in three (3) protocols. Resectable Stage I tumors are randomized (double blind trial) to intrapleural BCG + INH x12 weeks versus placebo intrapleural BCG + placebo INH x12 weeks. Resectable Stage II and III epidermoid carcinoma is randomized to observation versus radiation to tumor bed and mediastinum versus radiation as above + levamisole. Stage II and III adenocarcinoma and large cell carcinoma are randomized to a three drug combination of cytoxan, adriamycin, and cis-platinum versus intrapleural BCG + levamisole. An attempt will be made to correlate disease course with certain immune parameters before, during and after surgery.

CALIFORNIA, UNIVERSITY OF (LOS ANGELES) (N01-HB6-2971)

This contract is concerned with clinical studies to determine the efficacy of granulocyte transfusions and in what patient population they are useful.

CAPE TOWN, UNIVERSITY OF (N01-CM3-3738)

This contract has been designed to conduct eradicative clinical trials of patients with bronchogenic carcinoma, explore new and promising avenues of therapy for lung cancer, and devise and implement therapeutic protocols that will lead to a systematic and progressive understanding of the disease and promote its control. During the past contract year new drugs and new drug combinations have been explored in patients with distant metastases.

CATHOLIC MEDICAL CENTER OF BROOKLYN & QUEENS, INC. (N01-CM6-7081) (FORMERLY N01-CM2-3703)

This contract was initiated to provide an experimental mouse model for human breast cancer therapy. The purpose of the contract is to (1) test drugs to find agents with effectiveness against breast carcinoma, (2) study the influence of treatment schedules on "slow-growing" tumors, (3) study drug combinations, and (4) study drugs combined with surgery and/or immunotherapy. CD8F1 hybrid mice bearing spontaneous breast tumors are used in the surgical adjuvant and combined modality studies. Drug screening studies are performed in CD8F1 mice bearing 24-hour or advanced first generation tumor transplants. Agents to be screened are selected according to the following criteria: activity in the P388 screen, biochemical rationales, activity reported in the literature, candidates for clinical trial, and agents with established clinical activity.

CENTRAL DRUG RESEARCH INSTITUTE (PL-480 - AGREEMENT NO. NIH-01-004-1)

The objectives of this broad-based synthetic program are (a) the modification of natural products with anticancer activity, for example, alkaloids, antibiotics, peptides, etc., (b) synthesis of nitro aromatics and heterocyclics, and (c) synthesis of compounds related to the structural unit in emetine identified as a protein synthesis inhibitor. In addition, native Indian medicinal plants are collected, extracted, and fractionated for antitumor evaluation. All materials are evaluated according to NCI screening protocols.

CENTRAL DRUG RESEARCH INSTITUTE (PL-480 - AGREEMENT NO. NIH-01-004-N)

The Central Drug Research Institute, Lucknow, India, collects native Indian medicinal plants, supplies extracts for screening by the Developmental Therapeutics Program, and fractionates the active extracts. An active synthesis program for novel nitrogen heterocyclic compounds, excluding analogs of natural products, is a component of this contract. Excellent instrumentation is provided.

CHARLES RIVER BREEDING LABORATORIES, INC. (N01-CM5-0598) (FORMERLY NIH-75-C-598)

This procurement contract is designed to furnish 312,000 six-week-old CD2F1 (BALB/c female x DBA/2 male) hybrid mice free of pseudomonads for the Developmental Therapeutics Program contract studies. The breeding animals originate from the genetic center at this site.

CHARLES RIVER BREEDING LABORATORIES, INC. (N01-CM5-3812)

This primary genetic center has as its objective the development of germ-free foundation colonies of inbred rodents required for program studies. Pedigreed animals are derived via hysterotomy and foster-suckled in germ-free isolators.

CHARLES RIVER BREEDING LABORATORIES, INC. (N01-CM5-3812) (CONTINUED)

Selected pedigreed offspring are artificially contaminated with pure cultures of organisms and are developed as pedigreed expansion colonies in a variety of isolators. Offspring from this second stage are issued to secondary genetic centers which, in turn, produce breeding animals for large-scale production colonies. Classic methods for the maintenance of the animals are followed with respect to environmental controls and microbiological monitoring. A large-scale production colony is maintained in order to provide suitable numbers of rodents for laboratory investigators. Task 1 is concerned with the production of germ-free rodents, counterparts with defined microbial flora and large-scale production; Task 2 in Calco, Italy, produces rodents in biocontainment environments; and Tasks 3 and 4 are concerned with the production of rodents for specific investigations.

CHARLES RIVER BREEDING LABORATORIES, INC. (N01-CM5-3848)

This rodent production center contract supports a production effort designed to furnish animals as required by laboratory programs. Breeding animals are furnished by the Government.

CHEMICAL ABSTRACTS SERVICE (N01-CM4-3722)

The Chemical Abstracts Service (CAS) has participated in installing and is operating the new Chemical Information System in support of the Developmental Therapeutics Program accessioning and screening activities. This provides the improved capability to: (1) identify which accessions are new to the Program and which duplicate or are closely related to known active compounds; (2) produce computer-generated shipping documents and other communications (including structural diagrams) to suppliers, screeners, in-house staff, and the acquisition and storage and distribution contractors who are involved in the accessioning and screening process; (3) maintain inventory control; (4) maintain management and operational control of the accessioning process; and (5) search the files on-line to secure an immediate answer for a possible duplicate sample, for analogs of a compound of interest, or for a management inquiry. CAS is evaluating and installing various system enhancements which will increase the effectiveness and efficiency of searching, reporting, and correcting the file. Support is provided in the development of a model to preselect for acquisition compounds which would have a high probability of activity in the prescreen based on statistical evaluation of the contribution to activity made by the chemical structural fragments of compounds in the Program's collection and the biological activity of these compounds. Limited support is also provided in identifying for acquisition compounds in the literature which display biological characteristics which may correlate with antitumor activity.

CHESTER BEATTY RESEARCH INSTITUTE (N01-CM4-3736)

The principal objective of this project is focused on studies of (1) the growth of human tumors in immunologically deprived mice, and (2) the utilization of these xenograft systems as well as established transplantable mouse tumors for the detailed evaluation of drugs of clinical interest. Human tumors are grown in either CBA/Lac mice made immunologically incompetent by x-irradiation and thymectomy (T-lymphocyte depletion) or in athymic ("nude") mice. The Division of Cancer Treatment has identified the development of laboratory models for assessing chemotherapeutic effects against human tumor xenografts as a major problem objective. Tasks conducted under this contract are designed to define criteria for the successful xenografting of human tumors to animals utilizing patient resources of the Royal Cancer Hospital, London. The project is conducted in close collaboration with biochemical pharmacology studies supported by the Medical Research Council, Great Britain. In addition, this contract resource is used to acquire potentially effective new anticancer drugs from the British pharmaceutical and chemical companies and from universities; the Contractor's position in this regard is unique because the senior scientific staff serves as consultants to cancer chemotherapy investigators throughout Europe. Compounds so acquired are submitted to the Division of Cancer Treatment for antitumor screening.

CHICAGO, UNIVERSITY OF (N01-CM3-3748)

This contract is intended to support studies to isolate and compare the dihydrofolate reductase from normal and malignant tissues, primarily solid tumors, in order to determine any detectable molecular differences in these enzymes. The study also utilizes *in vitro* testing to help design inhibitors of human and subhuman dihydrofolate reductase. These are aimed at understanding the mechanism of resistance developed in human malignancy to antifols. This contract was phased out December 1976.

CHICAGO, UNIVERSITY OF (N01-CM6-7095)

This contract is designed to support prospective randomized, controlled protocol studies using combined modalities in the surgical adjuvant treatment of colon and rectal carcinoma.

CHILDREN'S HOSPITAL OF LOS ANGELES (N01-CM4-3803)

This contract is designed to conduct Phase I clinical studies with new anticancer drugs sponsored by the Division of Cancer Treatment, National Cancer Institute or to conduct Phase I clinical studies with new combinations or regimens mutually agreed upon. Phase I studies have been conducted on dianhydrogalactitol (NSC-132313), β-deoxythioguanosine (NSC-71851) and neocarzinostatin (NSC-157365) during the past year.

CHILDREN'S HOSPITAL MEDICAL CENTER (N01-CP6-5826)

As part of a multi-institutional cooperative effort, the effectiveness of routine nutritional practice will be compared with that of total parenteral nutrition via central-line hyperalimentation. Twenty-four pediatric patients with malignant tumors of the abdomen and/or pelvis requiring radiotherapy and intensive courses of multiple chemotherapeutic agents will be studied. Comparisons will be made with respect to: (a) tolerance to therapeutic schedule, (b) therapeutic response to combined chemotherapy and radiotherapy, (c) effectiveness of having the bowel "at rest" with respect to effects of therapy on gastrointestinal function, (d) nutritional status of the patient, and (e) complications during therapy. It is hypothesized that maintaining good nutritional status through total parenteral nutrition during combined radiotherapy to the abdomen and intensive chemotherapy, will result in fewer complications, decreased hospitalization time, and better therapeutic response. This hypothesis will be tested by a prospective randomized study comparing the patients maintained by total parenteral nutrition with those on routine dietary intake.

CHILDREN'S HOSPITAL OF LOS ANGELES (N01-CM5-3831)

This contract is designed to investigate the role of protected environments and prophylactic antibiotics as adjuvants to the remission induction chemotherapy of stages III and IV neuroblastoma, rhabdomyosarcoma and other small cell sarcomas. Trials were recently begun in 1975 but patient accrual was insufficient to justify continuation of the contract. The study was terminated in April 1977.

CHILDREN'S HOSPITAL OF LOS ANGELES (N01-CP6-5828)

As part of a multi-institutional cooperative effort, the effectiveness of routine nutritional practice will be compared with that of total parenteral nutrition via central-line hyperalimentation. Thirty pediatric patients with malignant tumors of the abdomen and/or pelvis requiring radiotherapy and intensive courses of multiple chemotherapeutic agents will be studied. Comparisons will be made with respect to: (a) tolerance to therapeutic schedule, (b) therapeutic response to combined chemotherapy and radiotherapy, (c) effectiveness of having the bowel "at rest" with respect to effects of therapy on gastrointestinal function, (d) nutritional status of the patient, and (e) complications during therapy. It is hypothesized that maintaining good nutritional status through total parenteral nutrition during combined radiotherapy to the abdomen and intensive chemotherapy, will result in fewer complications, decreased hospitalization time, and better therapeutic response. This hypothesis will be tested by a prospective randomized study comparing the patients maintained by total parenteral nutrition with those on routine dietary intake.

CLINICA NEUROCHIRURGICA DELL UNIVERSITA (DI PAVIA, ITALY) (N01-CM6-7056)

The clinical data from brain tumor patients provided under this contract are used to evaluate the efficacy of therapy in both Phase II and Phase III clinical trials. Single agent treatment as well as combined modalities therapy are used as detailed in the protocols of the Brain Tumor Study Group. The Phase III trials evaluate chemotherapy, radiation therapy and combinations thereof (multimodality therapy) following definitive surgery in the treatment of malignant gliomas. New chemotherapeutic agents are also being tested in Phase II trials in order to make the selection of chemotherapeutic agents for future Phase III trials of brain tumor patients more meaningful. The acquisition of patients into these prospective, controlled, randomized studies permits a comparative analysis of the clinical course of the patient and the toxicity of the various therapeutic modalities utilized as well as insight into the biological characteristics of the disease entity being investigated.

COLLABORATIVE RESEARCH, INC. (N01-CM7-7071) (FORMERLY N01-CM6-0506)

The main objective of this contract has been the elucidation of the chemistry of the metabolic activation of cyclophosphoramide and the synthesis of analogs of the active metabolite. The design of new compounds is directed towards such things as tissue specificity, selective transport and detoxification and less myelosuppression while broadening the antitumor activity. The Contractor has developed a rational synthesis of one of the postulated intermediates, "aldophosphamide", which is a tautomer of the probable initial metabolic product.

COLORADO STATE UNIVERSITY (N01-CM1-2192)

The objective of this research-type contract is to investigate the pathogenesis of adriamycin/daunomycin-induced cardiotoxicity utilizing clinical, morphological and biochemical parameters. The rabbit appears to be the most promising species for assessing the cardiotoxicity of this class of anticancer agents. Studies have been designed to establish optimal dose and time schedules to provide the highest incidence and maximum consistency of cardiotoxicity. Adriamycin analogues are being screened for cardiotoxicity using the rabbit with the hope of finding one with a maximum therapeutic effect and minimal cardiotoxicity. The rabbit is also being used for antidotal studies.
The Colorado contract is being phased out during this fiscal year.

COLORADO, UNIVERSITY OF, MEDICAL CENTER (N01-CM1-2186)

The purpose of this research and development contract is two-fold: (1) to study tRNA and mRNA methylases of normal and tumor tissue, to isolate and purify them to test natural and synthetic inhibitors of the enzymes from the two sources, and (2) to develop biochemical markers which stem from the turnover of tRNA and which are present in elevated levels in the blood and urine of patients with a variety of cancers. Accurate quantitative methods of measurement of four different markers have been developed including dimethylguanosine, methylinosine, pseudouridine and the unusually interesting marker, β-aminoisobutyric acid which can serve as a probe for the metabolism of DNA and transfer RNA from which it originates.

COMMUNITY BLOOD AND PLASMA SERVICE (N01-CM4-2150)

The purpose of this contract is to provide quadruplet units of HL-A typed platelets to patients in the NIH. The use of HL-A matched platelets decreases the risk of sensitization or production of antibodies to white cells and platelets in patients who require long-term hematologic support.

CONTROL DATA CORPORATION (N01-CM6-7107) (FORMERLY N01-CM3-3739)

The purpose of this contract is to provide data management and processing which enables simple and rapid retrieval of clinical information related to the patient data base provided by the participating group of contractors, the Brain Tumor Study Group, who enter patients on study according to specified protocols for treatment of brain tumors.

CORDOVA CHEMICAL COMPANY (N01-CM5-7031)

This service preparative contract provides for the resynthesis of a variety of compounds required for clinical or preclinical evaluation. The compounds prepared are not readily available on the open market or from the original supplier in the amounts required. The major effort of this contract is devoted to the preparation of large quantities of materials, in the multi-kilogram range, requiring pilot plant facilities.

DOT SYSTEMS, INC. (N01-CM5-3774)

This service contract was established to provide the necessary administrative and technical support to the Office of the Director, Division of Cancer Treatment. The primary function is the coordination and technical skills involved with the organization of conferences, graphic support, and editorial services. This contract terminated in FY '77 pending recompetition of expanded planning and analytical support services for DCT.

DOW CHEMICAL COMPANY (N01-CM2-3712)

This service-type contract is concerned primarily with the reproductive studies of three antineoplastic agents per year in accordance with the FDA Guidelines for Reproduction Studies for Safety Evaluation of Drugs for Human Use, dated January 1966. These studies are divided into three phases: 1) Phase I, general reproductive performance and fertility utilizing mice or rats of both sexes; 2) Phase II, teratology studies using mice and/or rats and rabbits; and 3) Phase III, perinatal studies employing mice and/or rats.

DOW CHEMICAL COMPANY (N01-CM5-3768)

This service preparative contract provides for the resynthesis of a variety of compounds required for clinical or preclinical evaluation. The compounds prepared are not readily available on the open market or from the original supplier in the amounts required. The major effort of this contract is devoted to the preparation of large quantities of materials, in the multi-kilogram range, requiring pilot plant facilities.

DUKE UNIVERSITY (N01-CM6-7010)

The clinical data from brain tumor patients provided under this contract are used to evaluate the efficacy of therapy in both Phase II and Phase III clinical trials. Single agent treatment as well as combined modalities therapy are used as detailed in the protocols of the Brain Tumor Study Group. The Phase III trials evaluate chemotherapy, radiation therapy and combinations thereof (multimodality therapy) following definitive surgery in the treatment of malignant gliomas. New chemotherapeutic agents are also being tested in Phase II trials in order to make the selection of chemotherapeutic agents for future Phase III trials of brain tumor patients more meaningful. The acquisition of patients into these prospective, controlled, randomized studies permits a comparative analysis of the clinical course of the patient and the toxicity of the various therapeutic modalities utilized as well as insight into the biological characteristics of the disease entity being investigated.

ELKINS-SINN, INC. (N01-CM6-7103)

The objectives of this contract are to provide facilities and capabilities for the development and production of parenteral investigational dosage forms for the Division of Cancer Treatment. The Contractor is responsible for observance of Good Manufacturing Practices and is responsible for completing all required analytical and safety tests on each product prepared.

ENERGY RESEARCH AND DEVELOPMENT ADMINISTRATION (Y01-CM1-0056)

The principal objective of this project is to study the cell cycle phase specificity of clinical drugs and drugs in development towards clinical trials. Drugs are evaluated for this ability to selectively inhibit progress through G_1, S, G_2 and M-phase of their proliferative cycle and for their selective cytocidal effects in these phases as well. Both synchronized and exponentially growing mammalian cell culture systems are used. The importance of such studies stems from the need to identify and develop drugs which may be specifically cytotoxic to non-proliferating but viable and clonogenic cells. The *in vitro* methodologies used were developed by the Contractor.

ENERGY RESEARCH AND DEVELOPMENT ADMINISTRATION (Y01-CM1-0060)

This contract involves the study of potential biologic markers for cancer in body fluids by means of high resolution high-pressure ion exchange chromatography. Initial efforts have focused on minor and methylated nucleic acid derivatives (tRNA catabolic end products) in urine. Sensitive analytical tools utilized in addition to high-pressure chromatography include gas chromatography, gas chromatography-mass spectrometry, and high- and low-resolution mass spectrometry. Preliminary quantitative results indicated elevated levels for specific nucleosides in the urine of approximately 60% of the cancer patients studied. Further efforts are focused on the analysis of specific serum protein bound sugars (utilizing high-pressure liquid chromatographic separation) in the blood of patients with malignancies. In addition, studies are in progress to determine the compounds represented by abnormal peaks obtained on chromatograms from patients with various selected types of cancer. This contract will terminate September 1977.

ENERGY RESEARCH AND DEVELOPMENT ADMINISTRATION (Y01-CM4-0101)

The objective of this effort is to develop a blood cell separation method which will collect sufficient granulocytes ($\sim 10^{10}$/day) from normal donors to permit widespread adoption of this technique in granulocyte replacement therapy. A unique design approach is being followed which was the end result of an extensive ORNL analysis. This consists of radically modifying the blood flow path of the existing IBM rotor to mechanically enhance rouleax formation and to photoelectrically monitor and control separating cell fractions in the interior of the rotor. This feasibility effort covers rotor fabrication, animal model testing and preliminary clinical trials to provide proven rotor designs adaptable to industrial product development and marketing interests.

ENERGY RESEARCH AND DEVELOPMENT ADMINISTRATION (Y01-CM4-0102)

This agreement furnishes instrumental and biological support to progressively adapt a prototype cell microfluorometer and computer data analysis system to human tumor cell characterization studies and drug evaluation projects planned by the National Cancer Institute. Engineering support has accomplished the design, testing and delivery of a multiparameter cell flow microfluorometer and sorting device coupled with a PDP-11/40 computer system. This FMF system is receiving progressive improvements through volume sensing devices, new software development, and special maintenance as a result of developing DCT needs. Biological investigations are concerned with FMF characterization of tumor growth as a function of tumor age or size.

FARMITALIA (N01-CM5-7014)

The major objective of this contract is the synthesis of analogs of adriamycin and daunomycin modified in both the aglycone and in the sugar moiety in order to extend the activity and decrease the toxicity (i.e., cardiotoxicity, myelosuppression, etc.). Modifications of the side chain of the aglycone should give active compounds with different pharmacological properties because an intercalation model shows the atoms of these side chains to be outside the DNA helix and, therefore, not to interfere with intercalation. Also structural modification in other parts of the aglycone is being explored. The purpose of the modifications of the sugar moiety is to reduce the enzymatic hydrolysis of the glycoside linkage.

FARMITALIA (N01-CM5-7036)

The thrust of this contract has been the development of a practical synthesis of the adriamycin aglycone and basic structural changes of the aglycone prior to the sugar coupling. The Contractor has synthesized related aglycones to reduce the cardiac toxicity of the parent compound. In addition to the synthesis of the aglycones, the contractor also couples selected aglycones with sugar moieties. This contract compliments the Farmitalia contract N01-CM5-7014.

FLOW LABORATORIES, INC. (N01-CM5-0571) (FORMERLY NIH-75-C-571)

This contract furnishes approximately 312,000 CD2F1 (BALB/c females x DBA/2 male) hybrid mice for compound evaluation studies. Breeding animals are furnished from genetic centers and/or rodent production colonies.

FLOW LABORATORIES, INC. (N01-CM6-7088)

This is a service contract to provide a clinical drug storage and distribution activity. The Contractor receives all incoming drug items from suppliers and manufacturers, stores them under optimal conditions and repackages them for subsequent shipment to clinical investigators throughout the world.

FRANKLIN INSTITUTE RESEARCH LABORATORIES (N01-CM5-3771)

This contract provides for the preparation, printing and distribution of Cancer Therapy Abstracts, Volumes 16 and 17 for 1975 and 1976. Computer tapes of the abstracts and indexes are also supplied for use in the ICRDB data base, Cancerline. The price of the annual subscriptions pays the costs of printing and mailing by the Contractor. This current awareness journal, published monthly, supplies abstracts and annotations of the world's literature pertinent to the treatment of cancer. Hard copy of the articles cited in the journal are also received from the Contractor. This contract will terminate June 1977.

FRANKLIN INSTITUTE RESEARCH LABORATORIES (N01-CM7-7092)

This contract provides for the preparation and furnishing of Cancer Therapy Abstracts, Volumes 18, 19 and 20 for the years 1977 through 1979.

FRED HUTCHINSON CANCER RESEARCH CENTER (N01-CM7-7148)

A member of the Lung Cancer Study Group: A collaborative group of six (6) institutions which are studying early non-oat cell lung cancer in three (3) protocols. Resectable Stage I tumors are randomized (double blind trial) to intrapleural BCG + INH x12 weeks versus placebo intrapleural BCG + placebo INH x12 weeks. Resectable Stage II and III epidermoid carcinoma is randomized to observation versus radiation to tumor bed and mediastinum versus radiation as above + levamisole. Stage II and III adenocarcinoma and large cell carcinoma are randomized to a three drug combination of cytoxan, adriamycin, and cis-platinum versus intrapleural BCG + levamisole. An attempt will be made to correlate disease course with certain immune parameters before, during and after surgery.

GEORGE WASHINGTON UNIVERSITY (N01-CM1-2206)

Investigations of physiological and biochemical variables in spontaneous lymphosarcoma and certain normal tissues in dogs are being conducted. The effects of methotrexate alone and in combination with calcium leucovorin on these variables are being studied. This contract has been completed and will terminate after its extension to May 31, 1977.

GEORGETOWN UNIVERSITY (N01-CM1-2123)

The objective of this program is to conduct virologic studies of patients participating in the National Cancer Institute's chemotherapeutic program to make possible evaluations that will aid the clinician in the selection of measures for supportive care and allow for the development of information of the importance of the role of viruses in the cancer patient in both protected and conventional environments.

GEORGETOWN UNIVERSITY (N01-CM3-3745)

The Eastern Cooperative Oncology Group consisted of 350 members and 35 cooperative institutions plus subsidiary hospitals. The Operations Office, funded by this contract, provides executive and administrative support for activities of the Group. On request from investigators, provides randomization and Group correspondence; obtains data from the investigators and maintains liaison with appropriate members of the National Cancer Institute staff. On renewal, the Operations Office provides similar services for the contract supported groups and institutions.

GEORGETOWN UNIVERSITY (N01-CM4-3732)

This contract has as its main objective the production of novel actinomycins by using biotransformation and co-metabolism fermentation techniques. These novel actinomycins will be produced in pure form in 100-500 mg. quantities and made available for broad-tumor screening. The use of many different compounds as substrates will be thoroughly explored.

GEORGETOWN UNIVERSITY (N01-CM6-7094)

This contract is designed to support prospective randomized, controlled protocol studies using combined modalities in the surgical adjuvant treatment of colon and rectal carcinoma.

GEORGETOWN UNIVERSITY (N01-CM6-7110)

This contract supports a comprehensive program for research in treatment and care of patients with gastrointestinal cancer. The program includes a fully staffed and functioning GI unit, with participation by the surgery, radiology, clinical, pathology, and medical departments. Specifically, the GI Research Program includes studies in: early detection, staging, new and established anticancer agents, combinations and multidisciplinary approaches, pharmacology, evaluation of markers, and professional training. A new major effort is devoted to the study of implications of nutritional deficiencies in cancer and efforts to correct them.

GRACE, W. R. (N01-CM6-7074)

This contract provides for the fractionation of confirmed active W. R. Grace fermentations. These fermentations were from various unique industrial substrates and presumptive chromatography indicates they are novel antineoplastic agents. Enough of the active material will be isolated to be thoroughly evaluated.

HARLAN INDUSTRIES, INC. (N01-CM5-0591) (FORMERLY NIH-75-C-591)

This contract furnishes approximately 312,000 six-week-old CD2F1 (BALB/c female x DBA/2 male) hybrid mice for compound evaluation studies. Breeding animals are furnished from genetic centers and/or rodent production centers.

HARLAN INDUSTRIES, INC. (N01-CM6-7079) (FORMERLY N01-CM2-3218)

This contract provides for the maintenance of a rodent production center. This center produces athymic (nude) mice for research and testing laboratory programs. All activities are performed in bio-containment environments.

HARVARD COLLEGE (N01-CM5-3825)

This project is based upon the exploration of the possibility that disarming malignant cells by increasing their immunogenicity by altering their cell surface polysaccharide composition, may be an effective chemotherapeutic approach. A detailed study of the effects of naturally occurring sugars and of sugar analogs on the oncogenicity of tumor cells and on the composition of their membrane glycoproteins, is being performed.

HAWAII, UNIVERSITY OF (N01-CM3-3747)

This contract provides for the collection of 3 to 5 lbs. of plant samples from among the indigenous and exotic higher plants of the Hawaiian Islands and Pacific area and the recollection of larger samples of those that are reproducibly active in the Developmental Therapeutics Program tests. Plants so obtained are shipped to the extraction contractor, WARF Institute, Inc., and the extracts tested at one of our contract screeners. Larger recollections are shipped to the individual fractionators as assigned by the Developmental Therapeutics Program.

HAZLETON LABORATORIES, INC. (N01-CM2-3701)

Mixed leukocyte cultures are an important determinant of the degree of compatibility of two individuals relative to transplantation antigens. The main objective is the immune evaluation of patients on chemotherapy using this technique. Because the bone marrow allograft transplant program has been terminated, this contract was phased out in March 1977.

HAZLETON LABORATORIES, INC. (N01-CM3-3708)

The major objectives of this contract are: (a) to obtain comparative data on the response of primates to known rodent carcinogens and to materials suspected of being carcinogenic in man; (b) to evaluate the long-term effects of antineoplastic agents which are being used clinically for long-term remissions and in the treatment of diffuse collagen disorders; (c) to obtain model tumor systems in primates; (d) to make available normal and tumor-bearing animals for pharmacologic, toxicologic, biochemical, and immunological studies; (e) to develop biological markers and diagnostic tests for detecting pre-neoplastic changes as well as frank neoplasia; and (f) to maintain a breeding colony of various species of primates so that these primates may be readily available for use. All work is carried out in close cooperation with the Laboratory of Chemical Pharmacology, NCI.

HAZLETON LABORATORIES, INC. (N01-CM4-3702)

This contract provides for the supply of dogs for leukocyte transfusion and autograft experiments. The specific experiments underway seek dose-response relationships in leukocyte transfusion of leukopenic dogs with pseudomonas sepsis, and examine the bone marrow stem cell dose, using CFU-C, to reconstitute hemopoiesis after marrow ablative chemo- or radiotherapy.

HAZLETON LABORATORIES, INC. (N01-CM5-7007)

This is a service contract for the in vivo testing of synthetic, crude materials of natural product origin, and fractions of previously confirmed active crude natural products. Testing is conducted according to Drug Evaluation Branch protocols at a rate of 25,000 L1210 equivalents per year. Crude natural products and new synthetic materials are prescreened in the P388 lymphocytic leukemia. Confirmed active materials may be tested in the tumor panel - exclusive of the xenografts and mammary tumor systems - upon request. Upon the request of members of the Drug Evaluation Branch, research and development work is conducted.

HAZLETON LABORATORIES, INC. (N01-CM6-0125) (FORMERLY NIH-76-C-0125)

This contract provides 300 beagle hounds for toxicologic assays. These animals are raised under controlled conditions in accordance with the specifications delineated in the contract. Breeding animals are supplied by the Contractor.

HAZLETON LABORATORIES, INC. (N01-CM6-7080) (FORMERLY N01-CM3-3705)

This contract supplies rapid and accurate clinical microbiologic information in support of patients at the Baltimore Cancer Research Center who receive cancer chemotherapy. It also provides patient microbiological surveillance cultures and environmental studies to afford a protected environment for patients at the BCRC and the Pediatric Oncology Branch at NIH. Preliminary data are teletyped to the Cancer Centers for use in patient care, and the final data is permanently stored in a computer. Retrieval of this information by computer techniques affords the data base for clinicians and research investigators. Microbiological report summaries are placed in the patients' permanent medical records.

HAZLETON LABORATORIES, INC. (N01-CM7-7126)

A contract is being advertised for award in FY'78 which will assist the intramural scientists in the Clinical Oncology Program in the storage and maintenance of laboratory animals. The facilities of the Clinical Center are extremely limited in the availability of space and personnel for laboratory animal handling, and this investigative resource is commonly available through a contract mechanism. In the past, this has been done ad hoc by individual Branches. The new contract proposes to combine the animal holding and transport needs of all Branches in Clinical Oncology into one contract facility. The Contractor will maintain, feed, and transport the animals but will not conduct research. Tumored animal models will also be provided.

HAZLETON RESEARCH ANIMALS, INC. (N01-CM7-7144)

This is a short-term contract in which a DNBA mammary tumor will be developed in the rat and used for the study of hormone sensitivity and chemotherapy interaction. At the present time, there is an ongoing clinical trial in patients with metastatic breast cancer in which the possibility that stimulation of breast cancer cell growth with hormones will render tumor cells more susceptible to the cytotoxic action of chemotherapy. It is very important that this clinical model be supplemented by pre-clinical data. The DNBA rat model is ideally suited for this purpose. This experiment is short-term and the study should be completed by January 1978.

HEALTH RESEARCH, INC. (N01-CM3-3726) (FORMERLY NEW YORK STATE DEPARTMENT OF HEALTH & HEALTH RESEARCH, INC.)

The objective of this contract is to develop antiviral-antitumor agents from a class of ansa macrolide compounds, the streptovaricin complex. These agents were first shown by the principal investigator to be reverse transcriptase inhibitors and to interfere with viral replication. A significant finding has been their later observation of relatively selective _in vivo_ effects, i.e., minimum cytotoxicity yet interference with viral replication. Streptovaricin complex fraction has been isolated and shown to inhibit spleen focus forming virus component of Friend virus at very low concentrations. Attempts are under way to isolate large quantities of this component to determine its structure and to carry out acute toxicity studies. Partial chemical structure of this compound has been determined by using a combination of nuclear magnetic resonances and mass spectrometric studies. This contract will terminate July 1977.

HEALTH RESEARCH, INC. (N01-CM3-3734) (FORMERLY NEW YORK STATE DEPARTMENT OF HEALTH & HEALTH RESEARCH, INC.)

This contract has been designed to conduct eradicative clinical trials of patients with bronchogenic carcinoma, explore new and promising avenues of therapy for lung cancer, and devise and implement therapeutic protocols that will lead to a systematic and progressive understanding of the disease and promote its control. During the past contract year, new drugs and new drug combinations have been explored in patients with distant metastases.

HEALTH RESEARCH, INC. (N01-CM4-3782) (FORMERLY NEW YORK STATE DEPARTMENT OF HEALTH & HEALTH RESEARCH, INC.

This contract is designed to explore in prospective randomized, controlled protocol studies (1) the value of adjuvant chemotherapy following a curative gastric resection, and (2) adjuvant therapy of locally advanced surgically incurable gastric carcinoma comparing single agent chemotherapy versus combination chemotherapy versus single agent plus radiotherapy.

HEALTH RESEARCH, INC. (N01-CM4-3785) (FORMERLY NEW YORK STATE DEPARTMENT OF HEALTH & HEALTH RESEARCH, INC.

The objective of this project is to develop a new test system, Profile of Drug Interactions. The approach to be used involves a determination in cell culture of the types of interaction (i.e., synergistic, additive or antagonistic) between the new drug and a selected panel of known chemotherapeutic agents or metabolic effectors. The interaction profile pattern produced can then be compared with those of other antitumor drugs to detect similarities in mechanisms of action. This contract will terminate June 1977.

HEALTH RESEARCH, INC. (N01-CM4-3794) (FORMERLY NEW YORK STATE DEPARTMENT OF HEALTH & HEALTH RESEARCH, INC.

This contract is designed to evaluate a combined modality protocol employing radiotherapy and chemotherapy in the treatment of locally unresectable carcinoma of the pancreas.

HEALTH RESEARCH, INC. (N01-CM5-3834) (FORMERLY NEW YORK STATE DEPARTMENT OF HEALTH & HEALTH RESEARCH, INC.

This contract is designed to support prospective randomized, controlled studies using combination chemotherapy in the treatment of breast cancer.

HEALTH RESEARCH, INC. (N01-CM5-7034) (FORMERLY NEW YORK STATE DEPARTMENT OF HEALTH & HEALTH RESEARCH, INC.

This contract is designed to support prospective randomized, controlled protocol studies using combined modalities in the surgical adjuvant treatment of colon and rectal carcinoma.

HEALTH RESEARCH, INC. (N01-CM5-7039) (FORMERLY NEW YORK STATE DEPARTMENT OF HEALTH & HEALTH RESEARCH, INC.

The objective of this contract is to establish *in vivo* and *in vitro* immunoassay systems to be used to test the selective effects that various anticancer drugs may have on the immune response. The studies include evaluation of the immunosuppressive activity of the antineoplastic agents in order to aid in the selection of candidate drugs; evaluation of the immunostimulating properties of various agents for possible application in cancer therapy; and determination of the effect of drugs on the antigenic properties of normal and tumor tissues in order to assess any influence on chemotherapeutic response.

HEALTH RESEARCH, INC. (N01-CM6-7114)

This contract represents a consortium of clinical centers studying the classification of non-Hodgkin's lymphoma. Because of the plethora of pathologic classifications in this area, the objectives of this collaborative effort are to collect and analyze retrospectively over 1000 cases of non-Hodgkin's lymphoma and correlate the clinical features (and lymphoid markers where available) with various pathologic classifications, seeking the major histologic determinants of clinical behavior. These objectives should be met in a two year contract period. This contract was phased out in January 1977.

HEALTH RESEARCH, INC. (N01-CM6-7117)

A collaborative group whose main thrust is to study various post-surgical adjuvants in early epithelial tumors of the ovary. A joint protocol for good risk Stage I (i.e., FIGO Stage IAi and IBi) compares observation to L-PAM post-operatively. A secondary study involves a histo-pathologic study of the incidence of unsuspected pelvic and/or paraortic node metastases in this patient population. A further joint protocol for other early disease (i.e., FIGO Stage IAii, IBii, IC, and II) separates patients according to presence or absence of macroscopic residual disease post-operatively. Patients with macroscopic residual disease are randomized to L-PAM versus L-PAM + pelvic radiotherapy. Patients without macroscopic residual disease are randomized to L-PAM versus intraperitoneal instillation of 15 mc of radioactive chromic phosphate.

HEALTH RESEARCH, INC. (N01-CM7-7101) (FORMERLY NEW YORK STATE DEPARTMENT OF HEALTH & HEALTH RESEARCH, INC. N01-CM0-2035)

This contract is designed to monitor and maintain genetic control of tumor strains and inbred mouse stocks. This service was established to assure continuous control of the biological materials used in program studies. The Contractor carries out the service by: (1) performing iso-antigenic typing tests of tumors used in the various screening tests, enabling the Developmental Therapeutics Program to maintain and distribute tumors identical to the prototype; (2) developing techniques and material for antigenic typing of tumors new to the screening program; (3) determining strain specificity of tumors originating in the inbred mouse; and (4) performing skin grafts and antigenic studies of mouse strains to assure their continuous genetic integrity and histocompatibility with other sublines maintained in counterpart genetic production centers.

HEBREW UNIVERSITY (HADASSAH MEDICAL SCHOOL) (N01-CM1-2127)

This research contract is concerned with the effects of an extract, MER (methanol extractable residue), obtained from attenuated tubercle bacilli (BCG) in stimulating the immune mechanism in animals and man with particular emphasis on antitumor activity. Clinical immunotherapy trials have been underway in patients with acute leukemia. This contract is now being phased out since the clinical trial has been completed.

HEM RESEARCH (N01-CM6-7066)

The main objectives of this contract are to provide stocks of cells producing candidate human type-C RNA tumor virus, and cells, viruses, and viral components. Human tumor specimens were analyzed for viral RNA sequences, using competitive molecular hybridization and also by examining the properties of RNA synthesized by blood cells maintained in short-term tissue culture.

IIT RESEARCH INSTITUTE (N01-CM4-3755)

This represents a support services contract to provide assistance to the Drug Evaluation Branch staff in preliminary data evaluation and follow-up on materials found active in the pre-screen. The Contractor participates with staff in monitoring testing and in expediting work on those materials which are recommended for testing in the tumor panel. Assistance is also provided by the design and maintenance of data files used as management tools by the Developmental Therapeutics Program staff.

IIT RESEARCH INSTITUTE (N01-CM5-3829)

This Contractor performs bacteriologic diagnostic services with emphasis upon the detection of *Salmonella spp*. and *Pseudomonas spp*. in program rodents and other materials used for animal husbandry practices. Specimens are forwarded by contract laboratories and animal production establishments. The scope of performance of this contract was changed recently to the extent that they are now monitoring animals leaving the genetic and rodent production centers primarily for the presence of ecto- and endoparasites. They also forward blood serum samples to the Microbiological Associates where these same animals are monitored for virus diseases. The results of these monitoring studies will be utilized to upgrade the quality of animals being sent to the laboratories. Diagnostic procedures are also carried out at this facility when disease problems occur in the various animal breeding colonies or in the laboratories using these animals.

IIT RESEARCH INSTITUTE (N01-CM5-7004)

This contract provides for the performance of *in vivo* testing of synthetic materials and natural products. Screening is being conducted according to Drug Evaluation Branch protocols at a level of approximately 50,000 tests

IIT RESEARCH INSTITUTE (N01-CM5-7004) (CONTINUED)

per year. New natural products and synthetics are being tested in the P388 leukemia in the mouse host. Tumor panel testing is conducted in all panel systems with the exception of the xenografts. Methodology and development are carried out only at the request of the Project Officer and during this year has been devoted to the development of the tumor panel test systems.

IIT RESEARCH INSTITUTE (N01-CM6-3832)

This contract provides for cytotoxicity screening in KB, P388, and L1210 cell cultures. Standardized assays are performed in accordance with procedures described by the Developmental Therapeutics Program protocols primarily as an aid in isolation and purification of potential antitumor agents from natural plant or animal sources. Selected synthetic materials are also screened as required by the Program. The Contractor may also participate in occasional methodology studies at the request of the Project Officer.

ILLINOIS, UNIVERSITY OF (N01-CM6-7090) (FORMERLY N01-CM2-2078)

This contract provides for the fractionation of confirmed active plant extracts and fermentation products in an attempt to isolate in a pure state and identify the active compound(s). Plant materials used in this work are obtained, for the most part, through the United States Department of Agriculture.

INDIAN CANCER RESEARCH INSTITUTE (PL-480 - AGREEMENT NO. NIH-01-010-1)

This PL-480 Agreement is for the collection and identification of natural products indigenous to India, the preparation of crude extracts from these materials, and the testing *in vivo* of these extracts as well as synthetic materials synthesized in India.

For this purpose, an *in vivo* screening laboratory is maintained in Bombay, India, together with an animal breeding colony to supply the necessary host animals. Currently, the P388 lymphoid leukemia is utilized with testing conducted as per current Developmental Therapeutics Program protocols. The P388 tumor system is employed for the testing of natural products, fractions and synthetics.

INDIANA UNIVERSITY FOUNDATION (N01-CM6-7057)

The clinical data from brain tumor patients provided under this contract are used to evaluate the efficacy of therapy in both Phase II and Phase III clinical trials. Single agent treatment as well as combined modalities therapy are used as detailed in the protocols of the Brain Tumor Study Group. The Phase III trials evaluate chemotherapy, radiation therapy and combinations thereof (multimodality therapy) following definitive surgery in the treatment of malignant gliomas. New chemotherapeutic agents are also being tested in

Phase II trials in order to make the selection of chemotherapeutic agents for future Phase III trials of brain tumor patients more meaningful. The acquisition of patients into these prospective, controlled, randomized studies permits a comparative analysis of the clinical course of the patient and the toxicity of the various therapeutic modalities utilized as well as insight into the biological characteristics of the disease entity being investigated.

INFORMATION PLANNING ASSOCIATES, INC. (N01-CM7-7104)

Information Planning Associates, Inc., provides technical assistance and support services in the area of investigational drug regulations. Information is gathered and assembled for the preparation of Investigational New Drug Applications (INDA's). This includes screening information, animal toxicology, chemistry, bibliographic information, drug labeling and the clinical protocol. This information is submitted to the Food and Drug Administration. Once an INDA is established, this Contractor maintains the files by amending information as necessary.

INSTITUT JULES BORDET (N01-CM5-3840)

The objective of this contract is the maintenance of a liaison office at the Institut Jules Bordet, Cancer Center of the University of Brussels, Belgium. The program is designed to foster close collaboration between European and United States investigators in the development and application of new clinical anticancer drugs and in the exchange of preclinical experimental and clinical scientific knowledge and materials requisite for maximum progress in cancer chemotherapy. In addition to the information activities, an animal tumor test is maintained for the more detailed evaluation and development of selected new compounds, in accordance with National Cancer Institute interests.

INSTITUT JULES BORDET (N01-CM5-7040)

This is a service contract for the *in vivo* screening of new synthetic and natural product materials and the follow-up testing of active materials. The primary screening is carried out in accordance with Developmental Therapeutics Program protocols. New materials are tested against the P388 leukemia test system. As requested, testing is also carried out in the panel of antitumor test systems. Detailed testing, such as schedule dependency studies in the active tumor system, is conducted with materials of potential clinical interest. Methodology studies, such as the characterization and evaluation of tumor test systems, are carried out as directed by the Project Officer. The current level of testing is approximately 25,000 L1210 equivalent tests per year.

INSTITUTE OF CANCER RESEARCH, ROYAL CANCER HOSPITAL (N01-CM2-3717)

The objectives of this contract are to study the effects of chemotherapeutic agents in experimental solid tumor systems and in human xenografts transplanted into immune-suppressed mice in relation to their proliferative state and their vascular supply, and compare the effects of these agents on the hematopoietic system. In the past, quantitative in vivo and in vitro clonogenic assays were developed for B16 melanoma and Lewis lung carcinoma and for normal marrow. More recently, in vivo diffusion chamber assays have been developed which will permit comparisons of clonogenic cell populations in mouse and human normal and tumor tissues in the same experimental system. Interrelationships among the effects of nitrosoureas, alkylating agents and radiation have been studied. Studies are in progress comparing cell survival in tumor and marrow following other drugs in single and multiple doses and in two-drug combinations. Radiotracer methods have been developed to measure tumor blood supply. These methods will be used to study proliferative and drug response characteristics in tumors in relation to their blood supply. This contract terminated December 1976.

INSTITUTO NAZIONALE DE LA CURA DEI TUMORI (N01-CM6-7115)

This contract represents a consortium of clinical centers studying the classification of non-Hodgkin's lymphoma. Because of the plethora of pathologic classifications in this area, the objectives of this collaborative effort are to collect and analyze retrospectively over 1000 cases of non-Hodgkin's lymphoma and correlate the clinical features (and lymphoid markers where available) with various pathologic classifications, seeking the major histologic determinants of clinical behavior. These objectives should be met in a two year contract period.

IOWA, UNIVERSITY OF (N01-CM4-3743)

The objectives of this contract are to develop, manufacture and package parenteral dosage forms of investigational drugs for clinical evaluation. This Contractor also has the capability to produce products for oral use, if required. The Contractor is responsible for completing required analytical and safety tests on each product prepared. All products produced are packaged, labeled and shipped to the National Cancer Institute for subsequent redistribution to clinical investigators.

IOWA, UNIVERSITY OF (N01-HB6-2973)

This contract is concerned with clinical studies to determine the efficacy of granulocyte transfusions and in what patient populations they are useful.

ISTITUTO NAZIONALE PER LO STUDIO E LA CURA DEI TUMORI (N01-CM3-3714)

This contract is an outstanding one in that it involves a multidisciplinary approach to several major tumors. In carcinoma of the breast, controlled treatment regimen protocols for combination chemotherapy plus surgery and a protocol for a multiple drug combination therapy are in progress. In brain tumors, surgery followed by radiation therapy and chemotherapy will continue to be evaluated in a controlled manner. In advanced adenocarcinoma of the colon and rectum, two combined chemotherapy protocols are in progress. The protocols for breast carcinoma and brain tumors are original in their details and are not being performed in any other institution. The gastrointestinal carcinoma protocols have been provided by the NCI's Gastrointestinal Tumor Study Group (GITSG) and are performed in conjunction with other GITSG members in the United States. The Division of Cancer Treatment, NCI, puts a very high priority on combined modality approaches to cancer therapy and to the testing of new chemotherapeutic regimens involving combinations of drugs. The protocols which are being followed are important to the efforts of the Division of Cancer Treatment in this area. Additional protocols will be added in the future.

ISTITUTO NAZIONALE PER LO STUDIO E LA CURA DEI TUMORI (N01-CM4-3726)

This contract represents utilization of a unique resource of both advanced and early patients with malignant melanoma for various controlled clinical trials. The Project Director is the Chairman of the International Group for the Clinical Study of Melanoma. There are 27 collaborating institutions including Eastern and Western Europe, Australia (Sydney), and South America (Montevideo). Investigators have surgical, pathologic, chemotherapeutic and immunotherapeutic expertise. A clinical trial to determine the value of adjuvant DTIC, BCG, DTIC + BCG or no further therapy following lymph node dissection in patients with nodal melanoma metastases or stage I lesions of the trunk is currently in progress. The study will determine whether the above adjuvant therapies increase the disease free interval for those patients. In addition, a trial of DTIC, DTIC + BCG, or DTIC + C. parvum in advanced malignant melanoma is also in progress. This study will determine the response rates and duration of response for patients with metastatic melanoma.

JACKSON LABORATORY (N01-CM6-0176) (FORMERLY NIH-263-76-C-0176)

This service contract provides for the procurement of approximately 52,000 AKR inbred mice to screening contractors for tumor transplantation and as breeders for large-scale hybrid production colonies. Breeding animals are supplied by the Contractor.

JAPANESE FOUNDATION FOR CANCER RESEARCH (N01-CM2-2054)

The objective of this contract is the maintenance of a liaison office at the Japanese Foundation for Cancer Research in Tokyo. The program is designed to foster close collaboration between Japanese and United States investigators in the development and application of new clinical anticancer drugs and in the exchange of preclinical experimental and clinical scientific knowledge and materials requisite for maximum progress in cancer chemotherapy. A small testing facility is also maintained for the screening and further evaluation of selected new compounds.

JOHNS HOPKINS UNIVERSITY (N01-CM4-3718)

The synthetic and catabolic rates of specific myeloma paraprotein will be studied utilizing radioactive guanidoarginine for labelling purposes in mice bearing a transplanted myeloma cell line. Efforts will be made to correlate total cell number with the above parameters in an attempt to work out a possible method for determining this number. Comparison will be made to an existing system which requires culture of the involved cells to determine synthetic rate.

KANSAS, UNIVERSITY OF (N01-CM1-2184)

The principal objective of this contract is to collect pharmacokinetic data on new and established antitumor agents in patients undergoing treatment for malignant disease and to analyze these data for individual variability which can be correlated with clinical response or some other pharmacologic parameter. Methotrexate, 5-fluorouracil, and dianhydrogalactone are currently under study.

KANSAS, UNIVERSITY OF (N01-CM2-3217)

This contract provides research capabilities in the area of formulation design and development. Formulation problems not ammenable to the usual solubilization and/or stabilization methods are investigated. The Contractor has particular expertise in application of molecular complexes and reversible derivatives to improve solubility. Pilot batch preparation and chemical evaluation of these novel formulations are carried out under this contract.

KANSAS, UNIVERSITY OF (N01-CM4-3802)

This contract is designed to conduct Phase I clinical studies with new anticancer drugs sponsored by the Division of Cancer Treatment, National Cancer Institute or to conduct Phase I clinical studies with new combinations or regimens mutually agreed upon. Phase I studies have been conducted on dianhydrogalactitol (NSC-132313), anguidine (NSC-141537), and chlorozotocin (NSC-178248) during the past year.

KANSAS, UNIVERSITY OF (N01-CM5-3819)

This Contractor maintains a genetic production center. Its most important function is to secure the maintenance of the strains of rodents that are of interest in Developmental Therapeutics Program investigations. Its spectrum includes three strains: C57BL/6, DBA/2, and BSVR/Sr. Animals not required for perpetuation of pedigree are supplied to screening contractors and as breeders for production colonies. Hybrids for special studies are produced as required for specific studies.

KENTUCKY, UNIVERSITY OF (N01-CM6-7038)

The detection of terminal deoxynucleotidyl transferase, an enzyme considered to be specific for thymus, in peripheral blood leukocytes of patients with acute lymphocytic leukemia (ALL) and chronic myelogenous leukemia (CML) in acute blast phase of the disease suggests that this enzyme can be useful as a diagnostic biological marker for following patients during treatment and in remission. The main objectives of this contract are: (1) to carry out analyses for terminal transferase on peripheral blood leukocytes and bone marrow from patients with ALL at diagnosis, during induction therapy and maintenance therapy, disease remission and in relapse, and (2) to obtain a correlation, if any, of the level of terminal transferase in ALL with cell surface markers and ultrastructure.

KENTUCKY, UNIVERSITY OF (N01-CM6-7058)

The clinical data from brain tumor patients provided under this contract are used to evaluate the efficacy of therapy in both Phase II and Phase III clinical trials. Single agent treatment as well as combined modalities therapy are used as detailed in the protocols of the Brain Tumor Study Group. The Phase III trials evaluate chemotherapy, radiation therapy and combinations thereof (multimodality therapy) following definitive surgery in the treatment of malignant gliomas. New chemotherapeutic agents are also being tested in Phase II trials in order to make the selection of chemotherapeutic agents for future Phase III trials of brain tumor patients more meaningful. The acquisition of patients into these prospective, controlled, randomized studies permits a comparative analysis of the clinical course of the patient and the toxicity of the various therapeutic modalities utilized as well as insight into the biological characteristics of the disease entity being investigated.

LABORATORY RESEARCH ENTERPRISES, INC. (N01-CM6-0124) (FORMERLY NIH-263-76-C-0124)

This contract provides 300 beagle hounds for toxicologic assays. These animals are raised under controlled conditions in accordance with the specifications delineated in the contract. Breeding animals are supplied by the Contractor.

LABORATORY SUPPLY COMPANY, INC. (N01-CM5-0577) (FORMERLY NIH-75-C-577)

This contract furnishes approximately 312,000 CD2F1 (BALB/c female x DBA/2 male) hybrid mice for compound evaluation studies. Breeding animals are furnished from genetic centers and/or rodent production colonies.

LABORATORY SUPPLY COMPANY, INC. (N01-CM5-3775)

This contract provides for the maintenance of a rodent production center. This center produces stocks and strains of rodents for research and testing laboratory programs. Production levels for individual colonies are correlated with requirements for specific investigations. This contract also furnishes breeding animals for large-scale production colonies. All activities are performed in bio-containment environments.

LEEDS, UNIVERSITY OF (N01-CM4-3727)

The purpose of this contract is the investigation of the usefulness of various "markers" in the study of gastrointestinal cancers with special reference to the earlier detection of metastases and their pattern of change during the evolution of the disease.

LEO GOODWIN INSTITUTE FOR CANCER RESEARCH (N01-CM5-3814)

This primary genetic center has as its objective the development of germ-free foundation colonies of inbred rodents. Pedigree animals are derived via hysterotomy and foster-suckled in germ-free isolators. Selected pedigreed offspring are artificially contaminated with pure cultures of non-pathogenic organisms and are developed as pedigreed expansion colonies in isolators. Offspring from this second stage are issued to secondary genetic centers which, in turn, produce breeding animals for large-scale production colonies. The methods commonly accepted as best practice are followed with respect to environmental controls and microbiological monitoring. A small-scale production colony is maintained in order to provide limited numbers of rodents for special research and testing studies.

LITTON BIONETICS, INC. (N01-CM1-2340)

The objectives of this project are (a) to purify viral antigens from RNA tumor viruses and human leukemic cells and to prepare antisera against these antigens, (b) to analyze the nucleic acid relatedness of DNA and RNA from human leukemic cells or of RNA from type-C viruses isolated from human cells to nucleic acids from known primate type-C viruses, (c) to study the effect of various elements, including growth factors and type-C viruses, on the growth and differentiation of hematopoietic stem cells, normal and leukemic, (d) to determine if viral-related macromolecules or other possible leukemia-specific macromolecules will be of value as diagnostic and prognostic tools for human leukemia, and (e) to expand basic research efforts in tumor cell biochemistry in animal model systems in order to achieve a better understanding of the neoplastic process and its prevention and treatment.

LITTON BIONETICS, INC. (N01-CM2-2062)

The main objective of this contract is the preparation, growth, and characterization of defined tissue culture cell lines and of fresh human leukemic blood cells. Cell cloning, cell genetics, cytochemistry, defining immunological cell markers and labeling cells with radioisotopic precursors for the isolation of metabolic products are also performed.

LITTON BIONETICS, INC. (N01-CM5-3778)

The Contractor performs bacteriologic diagnostic services with emphasis upon the detection of *Salmonella spp*. and *Pseudomonas spp*. in program rodents and other materials used for animal husbandry practices. Specimens are forwarded by contract laboratories and animal production establishments. Approximately 10,000 samples will be screened per year. The research portion of this contract (a minor component) is directed towards improvement in cultural techniques.

LITTON BIONETICS, INC. (N01-CM6-7067)

In the past year, a contract was let with Litton Bionetics under the direction of Dr. Steven Rosenberg for the purpose of immunological monitoring of patients undergoing immunotherapy. One of the major objectives of the Surgery Branch is to study immunotherapy in cancer, and several clinical protocols are underway. The immunoadjuvant effect of *C. Parvum* and BCG is being studied in patients with sarcomas, colon cancer, and malignant melanoma. In order to monitor the immunologic reactivity of these patients before, during and after treatment, it is important that a contract resource be available for the storage and cryopreservation of lymphocytes and serum for subsequent testing in patients. Because of the difficulties of day-to-day variability in many of the bioassays used to study the immune response, it is critical that these reagents be stored and cryopreserved in such a fashion as to allow simultaneous estimation of such assays as mitogen stimulation, immunoglobulin determinations, cellular cytotoxicity, and other tests of immunologic function. Further, the Contractor assists in the tracking and following of all patients on immunotherapy protocols for the purpose of correlating the clinical data with the immunologic findings.

LITTON BIONETICS, INC. (N01-CO2-5423)

The Government-owned, contractor-operated Frederick Cancer Research Center serves as a multi-faceted contract involving the following activities for the Division of Cancer Treatment:

Project 17

At the Frederick Cancer Research Center, the Division of Cancer Treatment has a fermentation pilot plant facility for the large-scale production and isolation of microbial products of interest to the program. Facilities are also available for the production of other natural products. A fermentation research laboratory has been completed and will be used to systematically evaluate various microbes' abilities to ferment unique substrates and produce novel compounds. The Division also participates in the operation of the Animal Farm and environmental control aspects of the program at FCRC.

Project 19

The Frederick Cancer Research Center was selected to establish, operate, and maintain a Biologic Markers Program under Project 19. The individual elements of the Program include an analytical services unit and specimen repository with computerized data storage/analysis capability, and a basic research unit for markers. The Program provides rapid routine analyses on established markers and conducts direct research to find, develop, and thoroughly explore the potentials of new markers for specific diseases, e.g., breast, gastrointestinal, lung, ovarian, brain, pediatric, etc., malignancies. Improvement of existing specific tumor markers will also be explored.

MADRID, UNIVERSITY OF (N01-CM5-3792)

The objective of this contract is to evaluate the effects of antitumor drugs on the electron transport processes in aerobic energy metabolism. The systems used are isolated heart and liver mitochondria, and intact Ehrlich ascites cells, using O_2 electrode measurements of oxygen consumption as well as spectroscopic methods to determine the oxidation state of electron transport chromophores. In addition, the action of selected drugs on myocardial membrane Na-K dependent ATPase is being evaluated.

MAKERERE UNIVERSITY COLLEGE COUNCIL (N01-CM7-1343)

This contract was developed to operate the Lymphoma Treatment Center and the Solid Tumor Center, collectively known as the Uganda Cancer Institute, to provide treatment and follow-up facilities for continued observation of patients with Burkitt's lymphoma, Hodgkin's disease, and other neoplasms. It is continuing under the direction of Dr. Sebastian Kyalwazi and Dr. Charles Olweny. This contract has been phased back to a level commensurate with appropriate follow-up of patients treated in the past, but does not support newly-initiated clinical trials.

"MARIO NEGRI" INSTITUTE OF PHARMACOLOGICAL RESEARCH (N01-CM3-3720)

The main purpose of this project is to establish a regional European contract as an extension of the Division of Cancer Treatment compound acquisition and screening program. The Contractor collects materials in Southern European countries and performs primary screening at a rate of approximately 7,500 L1210-equivalency tests per year.

"MARIO NEGRI" INSTITUTE OF PHARMACOLOGICAL RESEARCH (N01-CM5-3826) (FORMERLY ISTITUTO DI RICERCHE FARMACOLOGICHE "MARIO NEGRI")

This contract is for the purpose of development of immunochemotherapy studies on established or new experimental model systems. The studies include the development of new tumor-host systems in inbred as well as hybrid mice in various genetic combinations as well as studies of combined modalities of antineoplastic immunotherapy and chemotherapy.

"MARIO NEGRI" INSTITUTE OF PHARMACOLOGICAL RESEARCH (N01-CM6-7064) (FORMERLY ISTITUTO DI RICERCHE FARMACOLOGICHE "MARIO NEGRI" N01-CM2-3242)

Pharmacokinetic studies with adriamycin and various analogs are under investigation utilizing mice bearing solid tumors. Changes in pharmacokinetic parameters following multiple dose treatment are being explored as well as comparison of *in vitro* and *in vivo* drug effects. Particular emphasis is being placed on the derivative N-trifluoroacetyladriamycin-14-valerate.

MARSHALL RESEARCH ANIMALS, INC. (N01-CM6-0123) (FORMERLY NIH-263-76-C-0123)

This contract provides 300 beagle hounds for toxicologic assays. These animals are raised under controlled conditions in accordance with the specifications delineated in the contract. Breeding animals are supplied by the Contractor.

MARYLAND, UNIVERSITY OF (N01-CM4-3748)

Under this service contract, the University of Maryland at Baltimore provides suitable facilities, administrative support, ancillary support services and personnel for the inpatient operation of the Baltimore Cancer Research Center, as well as space for activities such as outpatient department, cell component therapy, patient care pharmacy and laboratory efforts necessary for the conduct of the program.

MASON RESEARCH INSTITUTE (N01-CM5-7003)

This service contract is for the *in vivo* testing of synthetic and natural products which are submitted to the Developmental Therapeutics Program.

MASON RESEARCH INSTITUTE (N01-CM5-7003) (CONTINUED)

The P388 tumor system is utilized for the testing of synthetic materials and natural products. In addition, testing is carried out on those materials assigned to tumor panel testing in the B16 melanoma, Colon 26, Lewis Lung and L1210 leukemia. Testing is proceeding at a level of approximately 37,000 L1210 equivalent tests per year. Schedule dependency studies to determine the optimal dose, schedule and route of administration, combination studies and special studies on other tumor test systems are conducted as directed by Drug Evaluation Branch staff.

MASON RESEARCH INSTITUTE (N01-CM5-7030)

New experimental solid tumor models will be developed and evaluated in order to improve predictive value of secondary screens in selecting drugs which will be active in human solid tumors. Emphasis will be placed on establishing human tumor xenografts in athymic nude mice, evaluating the biology of xenografts and developing methods for evaluating effects of drugs and other treatment modalities in this tumor model.

MASON RESEARCH INSTITUTE (N01-CM6-7011)

This contract supports the Clinical Oncology Program of the Division of Cancer Treatment with computer programming expertise for the development of clinical information systems and with data technician services for the maintenance and utilization of these systems. A wide variety of systems have been developed and are maintained for the clinical branches of the Division of Cancer Treatment.

MASSACHUSETTS INSTITUTE OF TECHNOLOGY (N01-CM4-3712)

The basic objective of this contract is the challenging synthesis related to the clinically effective antibiotic bleomycin; for example, the fragments of bleomycin. The difficult synthesis of key intermediates and of five unique fragments is progressing satisfactorily. The thrust now is to investigate and extend the synthesis of several of the fragments which show activity.

MAYO FOUNDATION (N01-CM0-2066)

The purpose of this contract is the investigation of new drugs in the treatment of advanced gastrointestinal tumors as a cooperative venture between the Mayo Foundation and the National Cancer Institute. The objective is to have each drug evaluated in a minimum of 20 cases of adenocarcinoma of the colon to meet the requirements of the Division of Cancer Treatment program linear array. Provision has been made for reporting of data on each trial in a manner which is consistent with the data retrieval currently employed intramurally at the National Cancer Institute. This will enable both direct comparisons and possible integration of data on new drugs from both of these sources.

MAYO FOUNDATION (N01-CM4-3783)

This contract is designed to explore in prospective randomized, controlled protocol studies (1) the value of adjuvant chemotherapy following a curative gastric resection, and (2) adjuvant therapy of locally advanced surgically incurable gastric carcinoma comparing single agent chemotherapy versus combination chemotherapy versus single agent plus radiotherapy.

MAYO FOUNDATION (N01-CM4-3796)

This contract is designed to evaluate a combined modality protocol employing radiotherapy and chemotherapy in the treatment of locally unresectable carcinoma of the pancreas.

MAYO FOUNDATION (N01-CM5-3772)

This contract is designed to support prospective randomized, controlled studies using combination chemotherapy in the treatment of breast cancer.

MAYO FOUNDATION (N01-CM5-3838)

This contract is designed to conduct Phase I clinical studies with new anti-cancer drugs sponsored by the Division of Cancer Treatment, National Cancer Institute or to conduct Phase I clinical studies with new combinations or regimens mutually agreed upon. Phase I studies on maytansine (NSC-153858), hycanthone (NSC-142982), rubidazone (NSC-164011) and chlorozotocin (NSC-178248) have been conducted during the past year.

MAYO FOUNDATION (N01-CM5-7033)

This contract is designed to support prospective randomized, controlled protocol studies using combined modalities in the surgical adjuvant treatment of colon and rectal carcinoma.

MAYO FOUNDATION (N01-CM5-7044)

This contract is designed to support Phase II-III studies in solid tumors, by means of prospective randomized, controlled clinical trials involving the use of combined modalities.

MAYO FOUNDATION (N01-CM6-7123)

A collaborative group whose main thrust is to study various post-surgical adjuvants in early epithelial tumors of the ovary. A joint protocol for good risk Stage I (i.e., FIGO Stage IAi and IBi) compares observation to L-PAM

MAYO FOUNDATION (N01-CM6-7123) (CONTINUED)

post-operatively. A secondary study involves a histo-pathologic study of the incidence of unsuspected pelvic and/or paraortic node metastases in this patient population. A further joint protocol for other early disease (i.e., FIGO Stage IAii, IBii, IC, and II) separates patients according to presence or absence of macroscopic residual disease post-operatively. Patients with macroscopic residual disease are randomized to L-PAM versus L-PAM + pelvic radiotherapy. Patients without macroscopic residual disease are randomized to L-PAM versus intraperitoneal instillation of 15 mc of radioactive chromic phosphate.

MAYO FOUNDATION (N01-CM7-7150)

A member of the Lung Cancer Study Group: A collaborative group of six (6) institutions which are studying early non-oat cell lung cancer in three (3) protocols. Resectable Stage I tumors are randomized (double blind trial) to intrapleural BCG + INH x12 weeks versus placebo intrapleural BCG + placebo INH x12 weeks. Resectable Stage II and III epidermoid carcinomas are randomized to observation versus radiation to tumor bed and mediastinum versus radiation as above + levamisole. Stage II and III adenocarcinoma and large cell carcinoma are randomized to a three-drug combination of cytoxan, adriamycin, and cis-platinum versus intrapleural BCG + levamisole. An attempt will be made to correlate disease course with certain immune parameters before, during and after surgery.

MEMORIAL HOSPITAL FOR CANCER AND ALLIED DISEASES (N01-CM4-3804)

This contract is designed to conduct Phase I clinical studies with new anticancer drugs sponsored by the Division of Cancer Treatment, National Cancer Institute or to conduct Phase I clinical studies with new combinations or regimens mutually agreed upon. Phase I studies on thiadiazole (NSC-4728), vindesine (NSC-245467), tetrahydrouridine and cytosine-arabinoside, and chlorozotocin (NSC-178248) have been conducted during the past year.

MEMORIAL HOSPITAL FOR CANCER AND ALLIED DISEASES (N01-CM5-3844)

This contract is designed to support Phase II-III studies in solid tumors by means of prospective randomized, controlled clinical trials involving the use of combined modalities.

MEMORIAL HOSPITAL FOR CANCER AND ALLIED DISEASES (N01-CM5-7019)

The clinical data from brain tumor patients provided under this contract are used to evaluate the efficacy of therapy in both Phase II and Phase III clinical trials. Single agent treatment as well as combined modalities therapy are used as detailed in the protocols of the Brain Tumor Study Group. The Phase III trials evaluate chemotherapy, radiation therapy and combinations thereof (multimodality therapy) following definitive surgery in the treatment of malignant gliomas. New chemotherapeutic agents are also being tested in Phase II trials in order to make the selection of chemotherapeutic agents for future Phase III trials of brain tumor patients more meaningful. The acquisition of patients into these prospective, controlled, randomized studies permits a comparative analysis of the clinical course of the patient and the toxicity of the various therapeutic modalities utilized as well as insight into the biological characteristics of the disease entity being investigated.

MEMORIAL HOSPITAL FOR CANCER AND ALLIED DISEASES (N01-CM5-7043)

This contract is for Phase II/III studies to detect useful therapeutic effects of new drugs alone and in various combinations in various solid tumors. The contract is in its first 19 months of performance under a 5 year contract. From July 1975 to February 1977, 1130 patients were studied in 19 approved protocols. A unique effort in testicular and in head and neck cancer has emerged from this contract.

MEMORIAL HOSPITAL FOR CANCER AND ALLIED DISEASES (N01-CP6-5829)

As part of a multi-institutional cooperative effort, the effectiveness of routine nutritional practice will be compared with that of total parenteral nutrition via central-line hyperalimentation. Eight pediatric patients with malignant tumors of the abdomen and/or pelvis requiring radiotherapy and intensive courses of multiple chemotherapeutic agents will be studied. Comparisons will be made with respect to: (a) tolerance to therapeutic schedule, (b) therapeutic response to combined chemotherapy and radiotherapy, (c) effectiveness of having the bowel "at rest" with respect to effects of therapy on gastrointestinal function, (d) nutritional status of the patient, and (e) complications during therapy. It is hypothesized that maintaining good nutritional status through total parenteral nutrition during combined radiotherapy to the abdomen and intensive chemotherapy, will result in fewer complications, decreased hospitalization time, and better therapeutic response. This hypothesis will be tested by a prospective randomized study comparing the patients maintained by total parenteral nutrition with those on routine dietary intake.

MIAMI, UNIVERSITY OF (N01-CM4-3800)

This contract is designed to evaluate a combined modality protocol employing radiotherapy and chemotherapy in the treatment of locally unresectable carcinoma of the pancreas.

MIAMI, UNIVERSITY OF (N01-CM5-7041)

The objective of this contract is to measure the immunosuppressive effects of drugs in order to determine how tissue and dose variables of treatment influence the depth and duration of suppression. These studies employ existing in vitro systems to determine the effect of the various antineoplastic agents on humoral and cell-mediated immune response.

MIAMI, UNIVERSITY OF (N01-CM6-7077)

This service contract laboratory conducts in vitro testing of synthetic materials (submitted in too small quantity for in vivo screening), crude materials of natural product origin, and fractions of confirmed natural products. Routinely, the before noted materials are tested in the 9KB system with testing in the P388 and/or L1210 system limited to those crude natural products that were active in vivo and not cytotoxic in the 9KB system. All tumor system testing is conducted according to Drug Evaluation Branch protocols at a level of 15,000 9KB test equivalents per year. Research and development testing is conducted at the request of the Project Officer.

MIAMI, UNIVERSITY OF (N01-CM6-7093)

This contract is designed to support prospective randomized, controlled protocol studies using combined modalities in the surgical adjuvant treatment of colon and rectal carcinoma.

MICHIGAN CANCER FOUNDATION (N01-CM5-3789)

The objective of this project is to characterize the range of molecular biological effects of antitumor drugs. In addition to effects on nucleic acid synthesis, the effects of new drugs on DNA structure, cell membrane permeability, cell surface electrical charge, cell surface adhesive properties and enzymes involved in the synthesis of cell surface glycoproteins will be studied.

MICHIGAN DEPARTMENT OF PUBLIC HEALTH (N01-CM3-3702)

This contract provides assay services, utilizing a variety of cell culture methods, as aids in the purification of potential antitumor agents from fermentation broths. The methods employed include paper chromatography and paper electrophoresis in conjunction with cell culture agar diffusion bioautography, as well as growth inhibition assays, both in stationary and suspension cultures. The related fermentation and isolation program services by this assay contract are financed by the Michigan Department of Health.

MICROBIAL CHEMISTRY RESEARCH FOUNDATION (N01-CM5-7009)

The major objective of this contract is the preparation of fermentations of marine, psychrophilic and thermophilic organisms. These fermentations are screened against various enzyme and other biochemical screens. Products are isolated which inhibit these various screens in sufficient quantities to be evaluated at the National Cancer Institute

MICROBIOLOGICAL ASSOCIATES, INC. (N01-CM4-3761)

This is a multifacet synthesis contract. The synthesis areas include exploration to determine the anticancer potential of analogs of alkaloids, antibiotics, amino acids and polyamines. The selection of projects is based in part on the quality of the investigator's rationale and the rapidity with which the synthesis can be implemented to provide products for anticancer testing. Thus, a large promising area of potential antitumor drugs is surveyed and any newly discovered active structural moieties investigated and exploited. This contract will terminate in early 1978.

MICROBIOLOGICAL ASSOCIATES, INC. (N01-CM4-3793)

This contract provides the National Cancer Institute with services for the storage and distribution of bulk chemicals and drugs. Samples are shipped to various domestic and foreign research laboratories. The contract provides for the weighing, packing and shipping of chemical samples and the maintenance of accurate inventory records.

MICROBIOLOGICAL ASSOCIATES, INC. (N01-CM5-3818)

This genetic production center supplies inbred rodents for tumor transplantation, for hybrid production, and for compound evaluation studies. Animals are supplied from a colony of two strains of rodents.

MICROBIOLOGICAL ASSOCIATES, INC. (N01-CM6-3810)

This contract resource assists in the measurement of leukocyte compatibility in clinical transfusions of leukopenic patients. The Contractor performs several leukoagglutinin assays and lymphocyte cross-match studies in order or insure safe and reliable blood component transfusions. These efforts are used in the daily management of the NCI leukopheresis. In addition, the Contractor operates computerized serum storage bank for all patients at the National Cancer Institute which has accessioned in excess of 20,000 samples.

MICROBIOLOGICAL ASSOCIATES, INC. (N01-CM6-7069)

This contract has as its major purpose serological diagnosis for the presence or absence of infectious mouse ectromelia, LCM, polyma, M. hepatitis, M. adenovirus, PVM, Sendai, Theiler's (GDVII), K, Rheo 3, SV5, Toolan's H-1, and the Rauscher and Moloney viruses. The Contractor also carries out ancillary research concerned with the effects of viruses, such as vaccinia and polyoma, on the growth of tumors; interaction of polyoma virus with tumors; antibody-producing capacity of tumor-bearing mice and the effect of the route of injection on antibody levels; and a comparison of the sensitivity of caesarian-derived and other mice to polyoma virus. This activity furnishes information on the effects of "passenger" agents on the growth of tumors used in the screen and on their effects on drug action. During a contract period of one year, this laboratory performs approximately 65,000 rodent serum and 6,000 tumor fragment diagnostic tests. The Contractor also prepares large quantities of vaccinia virus used for immunizing mice against infectious ectromelia.

MICROBIOLOGICAL ASSOCIATES, INC. (N01-CM7-7131)

The objective of this contract is to provide diagnostic viral studies, cultural, serological, and EM, on samples from patients who participate in the National Cancer Institute's chemotherapy program at the Baltimore Cancer Research Center and at the National Cancer Institute, Bethesda, Maryland. The results will not only aid the clinician in selecting optimal supportive care, but may also confirm the cause of obscure symptomatology and help clarify possible virus-bacterium interrelationships in immunosuppressed cancer patients.

MIDWEST RESEARCH INSTITUTE (N01-CM3-3722)

Midwest Research Institute is one of the two facilities where analyses of experimental medicinal compounds and their clinical formulations are performed for the Developmental Therapeutics Program. The Contractor determines the identity and purity of these materials, developing procedures for their analysis when standard methods are not available. The techniques employed include elemental analysis, chromatographic and spectrometric procedures, and other physiochemical procedures appropriate to the situation. This contract provides the necessary analytical data for filing with the Food and Drug Administration.

MIDWEST RESEARCH INSTITUTE (N01-CM3-3743)

Midwest Research Institute is one of our three major synthetic contractors. The primary objective of this contract is the rapid exploitation of leads generated by the Division of Cancer Treatment staff including Drug Evaluation Branch and various clinical groups. The current areas of investigation are the synthesis of alkoxy-phenanthridinium salts, analogs of natural products related to Coralyne and Nitidine, aminoanthraquinones, quinones, riboflavinlike analogs, alkoxybenz(g)isoquinolines, 7,8-benzochromones, and folic acid antagonists. The Contractor has developed and is applying techniques to reduce the carcinogenic properties of some polycyclic compounds and, at the same time, increase the antitumor properties.

MILES LABORATORIES (N01-CM4-3709)

This is a no-cost contract concerned with the marketing of a clinically useful drug, DTIC. The Contractor summarized the clinical and other data, prepared and filed the New Drug Application (NDA) with the Food and Drug Administration, and prosecuted the NDA. The NDA has been approved and the drug is currently on the market in the United States and in several other countries.

MINNESOTA, UNIVERSITY OF (N01-CM4-3788)

The major emphasis of this project is directed toward determining the cell uptake transport processes of selected antitumor drugs. In addition, the effects of membrane active, natural substances on the uptake and effectiveness of antitumor drugs are being evaluated.

MINNESOTA, UNIVERSITY OF (N01-CM6-7113)

This contract represents a consortium of clinical centers studying the classification of non-Hodgkin's lymphoma. Because of the plethora of pathologic classifications in this area, the objectives of this collaborative effort are to collect and analyze retrospectively over 1000 cases of non-Hodgkin's lymphoma and correlate the clinical features (and lymphoid markers where available) with various pathologic classifications, seeking the major histologic determinants of clinical behavior. These objectives should be met in a two year-contract period.

MINNESOTA, UNIVERSITY OF (N01-HB6-2972)

This contract is concerned with clinical studies to determine the efficacy of granulocyte transfusions and in what patient population they are useful.

MISSOURI, UNIVERSITY OF (N01-CM1-2323)

The objective of this contract is to develop procedures involving various advanced high resolution analytical techniques using specific instrumentation (e.g., GLC, amino acid analyzer, mass spectrometer, etc.) to measure quantitatively specific and potential biologic markers in the urine and blood of cancer patients and to utilize these procedures to study patients before, during and after therapy. Methods have been established for assay of polyamines (putrescine, spermidine, cadaverine, and spermine) and methylated nucleic acid derivatives, as well as other minor nucleic acid bases in urine and clinical studies are in progress to determine the value of these markers for following disease status in patients. In addition, preliminary work has been initiated for the assay of several other potential markers in serum and urine including serum fucose/protein ratio, urinary β-aminoisobutyric acid, and hydroxyproline/creatinine ratio.

MONSANTO RESEARCH CORPORATION (N01-CM2-3715)

This service preparative contract provides for the synthesis of radioactive labeled chemicals and drugs for use in preclinical pharmacological and clinical studies. Many of the materials prepared are not available from commercial sources. All materials, whether prepared at Monsanto or acquired from other sources, are analyzed for purity and identity by radioautography, assay, etc. This contract also provides storage facilities for labeled materials and distributes such as directed by the National Cancer Institute staff. This contract terminated December 1976.

MONSANTO RESEARCH CORPORATION (N01-CM6-7046) (FORMERLY N01-CM3-3709)

This service preparative contract provides for the large-scale synthesis of compounds required for preclinical and clinical studies. The compounds prepared are not readily available on the open market or from the original supplier in the amounts required. The major effort of this contract is devoted to the preparation of large quantities of materials, in the multi-kilogram range, requiring pilot plant facilities.

MONSANTO RESEARCH CORPORATION (N01-CM7-7140)

This service preparative contract provides for the large-scale extraction of 40,000 lbs. of *Maytenus* plant. The extracts then have the active antineoplastic agent maytansine removed and purified.

MONTEFIORE HOSPITAL (N01-CM6-7061)

The clinical data from brain tumor patients provided under this contract are used to evaluate the efficacy of therapy in both Phase II and Phase III clinical trials. Single agent treatment as well as combined modalities therapy are used as detailed in the protocols of the Brain Tumor Study Group. The Phase III trials evaluate chemotherapy, radiation therapy and combinations thereof (multimodality therapy) following definitive surgery in the treatment of malignant gliomas. New chemotherapeutic agents are also being tested in Phase II trials in order to make the selection of chemotherapeutic agents for future Phase III trials of brain tumor patients more meaningful. The acquisition of patients into these prospective, controlled, randomized studies permits a comparative analysis of the clinical course of the patient and the toxicity of the various therapeutic modalities utilized as well as insight into the biological characteristics of the disease entity being investigated.

MOUNT SINAI SCHOOL OF MEDICINE (N01-CM4-3703)

This contract provides 80 patients (previously untreated) with advanced ovarian cancer to study various drug combinations utilizing thiotepa, methotrexate, cytoxan, adriamycin and cis-platinum. Work to date has shown value of cis-platinum in combination with other agents and has verified Phase II data of Wiltshaw. Also performing *in vitro* testing of cis-platinum and studying use of sonography as a staging and follow-up procedure.

MOUNT SINAI SCHOOL OF MEDICINE (N01-CM5-3837)

This contract is designed to conduct Phase I clinical studies with new anti-cancer drugs sponsored by the Division of Cancer Treatment, National Cancer Institute or to conduct Phase I clinical studies with new combinations or regimens mutually agreed upon. Phase I studies on vindesine (NSC-245467), pyrazofurin (NSC-143095), and neocarzinostatin (NSC-157365) have been conducted in the past year.

MOUNT SINAI SCHOOL OF MEDICINE (N01-CM5-3841)

This contract is designed to explore in prospective randomized, controlled protocol studies (1) the value of adjuvant chemotherapy following a curative gastric resection, and (2) adjuvant therapy of locally advanced surgically incurable gastric carcinoma comparing single agent chemotherapy versus combination chemotherapy versus single agent plus radiotherapy.

MOUNT SINAI SCHOOL OF MEDICINE (N01-CM6-7096)

This contract is designed to support prospective randomized, controlled protocol studies using combined modalities in the surgical adjuvant treatment of colon and rectal carcinoma.

MURPHY BREEDING LABORATORIES, INC. (N01-CM5-0579) (FORMERLY NIH-75-C-579)

This contract furnishes approximately 312,000 six-week-old CD2F1 (BALB/c female x DBA/2 male) hybrid mice for compound evaluation studies. Breeding animals are furnished from genetic centers and/or rodent production centers.

NATIONAL ACADEMY OF SCIENCES (N01-CM5-3850)

This contract Task Order serves to develop standards for animal care and maintenance; shipping standards for the various species of laboratory animals; standards for nomenclature used to identify stocks and strains of laboratory animals; standards for methodology in maintaining axenic and gnotobiotic animals; standards for animal maintenance in the research laboratory; and laboratory animal procurement standards. These standards are formulated by ad hoc committees whose memberships represent commercial animal production colonies, governmental and academic institutions, commercial laboratories and non-profit research institutions.

NATIONAL ACADEMY OF SCIENCES (N01-CM5-7013)

This contract Task Order provides for conferences on diverse subjects that are intimately associated with the field of biomedical research. The agenda for specific meetings may be concerned with a common problem, or to develop the basis for a research program that is being considered by a segment of the scientific community. Conferences in the past have been held on research facilities, diseases of laboratory animals, professional training, the provision of animals for biological models for research, gnotobiotic technology, and requirements for standards in specific areas. Speakers are invited to present their thoughts and time is provided for open forum discussions. The proceedings of these conferences are published and made available to the scientific community.

NATIONAL NAVAL MEDICAL CENTER (Y01-CM5-0100)

The National Naval Medical Center has participated in programs furthering the understanding of the pathophysiology of breast cancer and in improving its treatment: (1) establishing a single multidisciplinary breast cancer clinic; (2) establishing joint therapeutic protocols in conjunction with those at the NCI; (3) involvement in joint laboratory studies relative to the detection and monitoring of breast carcinoma; and (4) publications and abstracts related to the contract. This contract also contributes to interdisciplinary efforts in testicular cancer and cooperates in the Eastern Cooperative Oncology Group protocols.

NAVAL WEAPONS CENTER (Y01-CM5-0101)

This project will develop and evaluate prototype instruments for the quantitation of growth in granulocyte precursor cells. The monitoring technique closely follows that developed in missile guidance systems for remote detection of small targets against complex bright backgrounds. The National Cancer Institute and National Heart and Lung Institute laboratories are collaborating in the design and evaluation of prototype instruments for ongoing Division of Cancer Treatment studies of leukopoietic mechanisms, the assessment of abnormalities in white cell proliferation, and the determination of marrow function associated with other diseases.

NEW ENGLAND MEDICAL CENTER (N01-CM6-7112)

This contract represents a consortium of clinical centers studying the classification of non-Hodgkin's lymphoma. Because of the plethora of pathologic classifications in this area, the objectives of this collaborative effort are to collect and analyze retrospectively over 1000 cases of non-Hodgkin's lymphoma and correlate the clinical features (and lymphoid markers where available) with various pathologic classifications, seeking the major histologic determinants of clinical behavior. These objectives should be met in a two-year contract period.

NEW YORK UNIVERSITY MEDICAL CENTER (N01-CM6-7059)

The clinical data from brain tumor patients provided under this contract are used to evaluate the efficacy of therapy in both Phase II and Phase III clinical trials. Single agent treatment as well as combined modalities therapy are used as detailed in the protocols of the Brain Tumor Study Group. The Phase III trials evaluate chemotherapy, radiation therapy and combinations thereof (multimodality therapy) following definitive surgery in the treatment of malignant gliomas. New chemotherapeutic agents are also being tested in Phase II trials in order to make the selection of chemotherapeutic agents for future Phase III trials of brain tumor patients more meaningful. The acquisition of patients into these prospective, controlled, randomized studies permits a comparative analysis of the clinical course of the patient and the toxicity of the various therapeutic modalities utilized as well as insight into the biological characteristics of the disease entity being investigated.

NORTHEASTERN UNIVERSITY (N01-CM5-3741)

The primary thrust of this contract is the development of central nervous system (CNS) penetrating antineoplastic agents, composed of an antitumor moiety and a known CNS active agent. Two groups of compounds, the aporphines and the aminophenyl-3-aminoisoquinolines, have been synthesized as the CNS penetrating portion of the molecule. These are incorporated with known alkylating functions. The contract is in its terminal year.

OHIO STATE UNIVERSITY RESEARCH FOUNDATION (N01-CM3-3736)

This contract was designed to conduct eradicative clinical trials of patients with bronchogenic carcinoma, explore new and promising avenues of therapy for lung cancer, and devise and implement therapeutic protocols that will lead to a systematic and progressive understanding of the disease and promote its control. During the past contract year new drugs and new drug combinations have been explored in patients with distant metastases. This contract terminated December 1976.

OHIO STATE UNIVERSITY RESEARCH FOUNDATION (N01-CM5-3828)

The purpose of this contract is to obtain information on the disposition and metabolism of new agents entering Phase I clinical trial. The Contractor has completed work on the pharmacologic disposition and metabolism of cytembena in man. They have identified several metabolites of the drug derived by successive degradation of the halo-side chain of the drug and have delineated multiphasic plasma pharmacokinetics in man. They have also demonstrated quantitative absorption of orally administered drug. In the coming year, attention will turn to the clinical pharmacokinetics and metabolism of a new agent, probably the plant alkaloid, maytansine.

OHIO STATE UNIVERSITY RESEARCH FOUNDATION (N01-CM5-7015)

The immediate objective of this contract is to synthesize fraudulent sugars related to daunosamine. Daunosamine is an integral part of the molecules of two antitumor agents, namely daunomycin and adriamycin. The design and synthesis of these daunosamine analogs and their coupling to selected aglycones are part of an overall plan to find anthracycline antitumor agents with less cardiotoxicity.

OHIO STATE UNIVERSITY RESEARCH FOUNDATION (N01-CM6-7060)

The clinical data from brain tumor patients provided under this contract are used to evaluate the efficacy of therapy in both Phase II and Phase III clinical trials. Single agent treatment as well as combined modalities therapy are used as detailed in the protocols of the Brain Tumor Study Group. The Phase III trials evaluate chemotherapy, radiation therapy and combinations thereof (multimodality therapy) following definitive surgery in the treatment of malignant gliomas. New chemotherapeutic agents are also being tested in Phase II trials in order to make the selection of chemotherapeutic agents for future Phase III trials of brain tumor patients more meaningful. The acquisition of patients into these prospective, controlled, randomized studies permits a comparative analysis of the clinical course of the patient and the toxicity of the various therapeutic modalities utilized as well as insight into the biological characteristics of the disease entity being investigated.

OKLAHOMA, UNIVERSITY OF (N01-CM6-7108)

The major objective of this contract is the preparation, from animal materials of marine species, of extracts suitable for bioassay in the National Cancer Institute antitumor screen. The extracts are prepared in accordance with procedures acceptable to the National Cancer Institute. Extracts showing preliminary activity are reconfirmed and those materials showing confirmed activity are assigned for fractionation and isolation.

ONTARIO CANCER INSTITUTE (N01-CM7-7152)

A member of the Lung Cancer Study Group: A collaborative group of six (6) institutions which are studying early non-oat cell lung cancer in three (3) protocols. Resectable Stage I tumors are randomized (double blind trial) to intrapleural BCG + INH x12 weeks versus placebo intrapleural BCG + placebo INH x12 weeks. Resectable Stage II and III epidermoid carcinoma is randomized to observation versus radiation to tumor bed and mediastinum versus radiation as above + levamisole. Stage II and III adenocarcinoma and large cell carcinoma are randomized to a three drug combination of cytoxan, adriamycin, and cis-platinum versus intrapleural BCG + levamisole. An attempt will be made to correlate disease course with certain immune parameters before, during and after surgery.

OREGON STATE UNIVERSITY (N01-CM2-3201)

The purpose of this contract is to acquire information on the disposition of drugs in animals so that such information is available prior to initiating clinical trials. Specifically, the three nitrosoureas, BCNU, CCNU and MeCCNU are being studied from two points of view. First, the metabolic alterations and chemical degradation that these drugs undergo are being studied in detail with a view toward the effects of these changes on their biological properties. Second, the effects of these agents on the ability of the host hepatic microsomes to metabolize other drugs are under investigation.

PARKE, DAVIS & COMPANY (N01-CM2-3708)

This service preparative contract provides for the resynthesis of a variety of compounds required for clinical or preclinical evaluation. The compounds prepared are not readily available on the open market or from the original supplier in the amounts required. About 50% of the effort on this contract is devoted to the preparation of large quantities of materials, in the multi-kilogram range, requiring pilot plant facilities.

PARKE, DAVIS & COMPANY (N01-CM7-7146) (FORMERLY N01-CM4-3754)

This fermentation contract is designed primarily to obtain novel antitumor agents. This contract includes: (1) the preparation of fermentation beers from various microbes isolated from unique substrates from various parts of the world and fermented under a bevy of environmental and stress situations; (2) an *in vitro* tissue culture assay laboratory which will assist in pre-screening fermentation broths for cytotoxicity and will be used to help assay chemical fractions, fermentation improvement samples and large pilot plant lots more quickly; (3) a small biotransformation program; (4) the isolation work required to obtain the active component from the confirmed active beers; (5) the production of large quantities of antineoplastic agents approved for clinical trials; and (6) the preparation of appropriate dosage forms of such agents.

PENNSYLVANIA, UNIVERSITY OF (N01-CM3-3719)

This contract provides for, on a multiphase basis, carrying out the design and installation of the Chemical Information System to handle a wide range of compound accessioning activities. This new system provides improved capability to: (1) identify which compounds are new to the Developmental Therapeutics Program, including the flagging of analogs and duplicates of compounds of interest; (2) produce computer-generated shipping documents and other communication (including chemical structural diagrams) to suppliers, screeners, in-house staff and contractors engaged in the acquisition, storage and distribution process; (3) maintain inventory control; (4) conduct on-line searches involving both structural and nonstructural information using simplified inquiry procedures and providing structure output; and (5) maintain management and operational control of the system. The system has been installed in the Chemical Abstracts Service (CAS) which is the Contractor operating the system. The final phase of this contract provides for completing the system documentation and training CAS programmers to assume the responsibility for system maintenance. This contract will terminate October 1977.

PENNSYLVANIA, UNIVERSITY OF (N01-CM5-3830)

The objective of this contract is to evaluate the acute and chronic effects of selected antitumor drugs on the energy performance status of isolated, perfused organs using both surface fluorescent and transmitted light absorbance methods developed by the Contractor. These models are to be applied to delineating the biochemical basis of either the therapeutic activity or limiting toxicity of selected drugs and their analogs.

PFIZER, INC. (N01-CM5-3805)

The main purposes of this contract are to produce large quantities of primate and putative human type-C viruses in tissue culture and to purify and concentrate these viruses from the tissue culture fluids. In addition, the Contractor is to prepare certain viral components both to use in quality control of virus production and to analyze the human tissues for the presence of viral internal antigens by competition radioimmunoassays.

PHARM-ECO (N01-CM2-3707)

This service preparative contract provides for the resynthesis of a variety of compounds required for clinical or preclinical evaluation. The compounds prepared are not readily available on the open market or from the original supplier in the amounts required. About 70% of the effort of this contract is devoted to the preparation of large quantities of material, in the multikilogram range.

PHILIPS ROXANE LABORATORIES, INC. (N01-CM6-7053) (FORMERLY N01-CM1-2139)

This service contract provides the Division of Cancer Treatment with facilities for development, formulation and production of oral dosage forms of investigational drugs. The dosage forms prepared at Philips Roxane Laboratories are packaged, labeled and shipped to the National Cancer Institute for subsequent distribution to clinical investigators.

POLYSCIENCES, INC. (N01-CM7-7070)

The major objective of this contract is to extract, isolate, and purify antineoplastic plant agents in large enough quantities for toxicology and clinical trials. In addition, in some cases, research into the obtaining of a practical large-scale isolation procedure will be done.

POMONA COLLEGE (N01-CM3-3703)

The objectives of this contract are: 1) the study of quantitative structure-activity relationships in the inhibition of dihydrofolate reductase by triazine antifols, and 2) the development of high-pressure liquid chromatography as a technique for the estimation of partition coefficients.

POMONA COLLEGE (N01-CM6-7062)

This contract conducts quantitative structure-activity studies on congeneric series of antitumor agents. In addition, the contract provides experimental determination of necessary physicochemical parameters (log P, E_s, sigma) when these values are not available in the literature.

PUBLIC HEALTH SERVICE, DIVISION OF FEDERAL HEALTH PUBLIC SERVICES (TRANSFER OF FUNDS - Y01-CM4-0003) (W. HYATTSVILLE, MARYLAND)

This Transfer of Funds permits service support of the National Cancer Institute, Intramural Baltimore Cancer Research Center Program. This includes suitable laboratory facilities, support personnel and other administrative support.

PURDUE UNIVERSITY (N01-CM6-7091)

This contract provides for the fractionation of confirmed active plant extracts in an attempt to isolate in a pure state and identify the active compound(s). Plant material used in this work is obtained, for the most part, through the United States Department of Agriculture.

RADIOBIOLOGICAL INSTITUTE, ORGANIZATION FOR HEALTH RESEARCH (N01-CM5-3763)

The objective of this contract is to conduct preclinical investigations of the combination modality of radiation and hypoxic radiosensitizers plus chemotherapy. Appropriate tumor test models are being explored and developed for this combination modality. With such models determination will be made of combinations and regimens of drug plus radiation that are optimal. The program is designed to provide an appropriate preclinical basis for the clinical application of radiation plus chemotherapy.

RCA RESEARCH LABORATORIES (N01-CM6-7098)

Studies directed to the development of a leadless tissue implanted temperature monitoring probe will be undertaken. The probe will be passively interrogated by a low level directional microwave signal employing sub-milliwatt power levels. The nature of the reflected signal will be determined by the local tissue temperature at the probe. Materials studied will be undertaken and supported by experimental feasibility investigations for the use of this technique to measure local tissue temperature.

RESEARCH FOUNDATION OF STATE UNIVERSITY OF NEW YORK (N01-CM3-3715)

The Contractor provides statistical and administrative support for the Gastrointestinal Tumor Study Group and phase-out of the Working Party for Therapy of Lung Cancer data files.

RESEARCH FOUNDATION OF STATE UNIVERSITY OF NEW YORK (N01-CM5-3766)

This contract is to perform *in vivo* and *in vitro* basic investigation and development of new experimental models for studying the effects of anticancer agents on immune responses. Specifically, the effects on T and B cells, macrophages and the *Hh* systems will be assessed.

RESEARCH TRIANGLE INSTITUTE (N01-CM6-7089) (FORMERLY N01-CM9-2019)

The primary purpose of this contract is to fractionate confirmed active plant extracts in an attempt to isolate and identify the active antineoplastic compound. In addition, active fermentation materials obtained from other contractors are isolated, purified and identified. The plants used for this work are primarily obtained from the United States Department of Agriculture. The Contractor has many plants which are or have been in some stage of fractionation and several fermentation crude materials which are being purified. Several of these active agents have been isolated and are being evaluated more broadly. A KB cell culture facility is used to expedite fractionation work.

RESEARCH TRIANGLE INSTITUTE (N01-CM6-7121)

This service preparative contract provides for the synthesis of radioactive labeled chemicals and drugs for use in preclinical pharmacological and clinical studies. Many of the materials prepared are not available from commercial sources. All materials, whether prepared at the Institute or acquired from other sources, are analyzed for purity and identity by radioautography, assay, etc. This contract also provides storage facilities for labeled materials and distributes such as directed by the National Cancer Institute staff.

RUTGERS STATE UNIVERSITY (N01-CM6-7106)

This contract provides for the isolation of unique Black Yeast and its polysaccharides in sufficient quantities for evaluation in the Drug Evaluation *in vivo* solid tumor tests.

SAINT JUDE'S CHILDREN'S RESEARCH HOSPITAL (N01-CM7-7127)

The main objective of this contract is to synthesize analogs which will inhibit the anabolic utilization of preformed pyrimidines. Cancer cells tend to use preformed pyrimidines scavenged from the blood for growth and development. This is in contrast to normal cells which synthesize required pyrimidines by the *de novo* path. The inhibitors prepared under the contract are expected to selectively destroy tumors with minimum effect on normal cells.

SIDNEY FARBER CANCER INSTITUTE (N01-CM4-3781)

This contract is designed to explore in prospective randomized, controlled protocol studies (1) the value of adjuvant chemotherapy following a curative gastric resection, and (2) adjuvant therapy of locally advanced, surgically incurable gastric carcinoma comparing single agent chemotherapy versus combination chemotherapy versus single agent plus radiotherapy.

SIDNEY FARBER CANCER INSTITUTE (N01-CM4-3799)

This contract is designed to evaluate a combined modality protocol employing radiotherapy and chemotherapy in the treatment of locally unresectable carcinoma of the pancreas.

SIDNEY FARBER CANCER INSTITUTE (N01-CM5-3839)

This contract is designed to conduct Phase I clinical studies with new anticancer drugs sponsored by the Division of Cancer Treatment, National Cancer Institute or to conduct Phase I clinical studies with new combinations or regimens mutually agreed upon. Phase I studies on maytansine (NSC-153858),

SIDNEY FARBER CANCER INSTITUTE (N01-CM5-3839) (CONTINUED)

neocarzinostatin (NSC-157365), high dose methotrexate-thymidine rescue and aminopterin - citrovorum factor rescue have been conducted in the past year.

SIDNEY FARBER CANCER INSTITUTE (N01-CM5-7035)

This contract is designed to support prospective randomized, controlled protocol studies using combined modalities in the surgical adjuvant treatment of colon and rectal carcinoma.

SIDNEY FARBER CANCER INSTITUTE (N01-CM6-7037)

The Contractor conducts Phase II/III studies in patients with solid disseminated tumors. The tumors to be included are limited to: lung, breast, melanoma, soft tissue and bone sarcoma. A minimum of 400 patients a year, with no less than 25 patients in any tumor type. These patients are treated intensively with chemotherapy either alone or in combination with radiotherapy, immunotherapy or surgery in protocols agreed upon by the Project Officer and Principal Investigator. Studies in nutritional support for patients undergoing systemic antineoplastic chemotherapy are also conducted under this contract. Major efforts in melanoma and in breast cancer have contributed to better regimens in the advanced stages of these tumors.

SIMONSEN LABORATORIES, INC. (N01-CM5-0578) (FORMERLY NIH-75-C-578)

This contract furnishes approximately 312,000 six-week-old B6D2F1 (C57BL/6 female x DBA/2 male) hybrid mice for compound evaluation studies. Breeding animals are furnished from genetic centers and/or rodent production centers.

SIMONSEN LABORATORIES, INC. (N01-CM5-3817)

The Contractor maintains a genetic production colony of seven inbred strains of rodents. Small quantities of animals from the colony are made available for tumor transplantation and the majority are furnished for large-scale production colonies.

SIMONSEN LABORATORIES, INC. (N01-CM5-3847)

This contract provides for the maintenance of a rodent production center. This center produces stocks and strains of rodents for research and testing laboratory programs. Production levels for individual colonies are correlated with requirements for specific investigations. This contract furnishes breeding animals for large-scale production colonies.

SLOAN-KETTERING INSTITUTE FOR CANCER RESEARCH (N01-CM5-3820)

The objective of this contract is to extend the techniques of in situ molecular hybridization to the presence of oncornavirus nucleic acid sequences in individual mammalian cells. To this end tumor virus nucleic acids labelled with ^{125}I to the highest possible specific activity are being tested for their usefulness for in situ hybridization, and techniques are being developed for the characterization of viral RNA by fingerprint analysis.

SMALL BUSINESS ADMINISTRATION (N01-CM4-3719)

The purpose of this contract is to provide supportive services in immunology, virology, and tissue culture to the cancer chemotherapy program. The immediate goals are: (1) to develop a sensitive in situ immunological assay system for detecting virus-related antigens in human leukemic cells as a potential diagnostic tool; (2) to perform other immunological tests including immunofluorescent tests and complement fixation tests for virus-related antigens in human leukemia cells and other pertinent cells; (3) to test various compounds available to Laboratory of Tumor Cell Biology investigators for their inhibitory effect on type-C virus biological activity and on human leukemic cells in vitro; and (4) to perform other specialized supportive procedures essential to LTCB investigators, including cytogenetic analyses, cell cloning, and virological procedures of biological interest.

SOUTHERN ANIMAL FARMS (N01-CM5-0599) (FORMERLY NIH-75-C-599)

This contract furnishes approximately 312,000 CD2F1 (BALB/c female x DBA/2 male) hybrid mice for compound evaluation studies. Breeding animals are furnished from genetic centers and/or rodent production colonies.

SOUTHERN ANIMAL FARMS (N01-CM5-3845)

This contract provides for the maintenance of a rodent production center. This center produces stocks and strains of rodents for research and testing laboratory programs. Production levels for individual colonies are correlated with requirements for specific investigations. This contract furnishes breeding animals for large-scale production colonies.

SOUTHERN CALIFORNIA, UNIVERSITY OF (N01-CM4-3795)

This contract is designed to evaluate a combined modality protocol employing radiotherapy and chemotherapy in the treatment of locally unresectable carcinoma of the pancreas.

SOUTHERN CALIFORNIA, UNIVERSITY OF (N01-CM5-3842)

This contract is designed to explore in prospective randomized, controlled protocol studies (1) the value of adjuvant chemotherapy following a curative gastric resection, and (2) adjuvant therapy of locally advanced, surgically incurable gastric carcinoma comparing single agent chemotherapy versus combination chemotherapy versus single agent plus radiotherapy.

SOUTHERN CALIFORNIA, UNIVERSITY OF (N01-CM6-7065) (FORMERLY N01-CM2-3241)

The purpose of this contract is to develop a detailed pharmacokinetic profile of clinically useful antineoplastic agents. Thus far, a comprehensive model has been developed for adriamycin distribution in the rabbit, utilizing a multicompartmental analysis. This model, appropriately modified for physiologic parameters in man, has been applied in preliminary fashion to plasma pharmacokinetics in man and proved to be predictive of parent drug concentrations in plasma. Further collection and analysis of human data are planned, as well as initiation of similar studies of cytoxan pharmacokinetics.

SOUTHERN CALIFORNIA, UNIVERSITY OF (N01-CM6-7084)

The main purpose of this contract is the synthesis of compounds designed to bind specifically to DNA and certain enzymes. The three specific objectives of this contract are: (a) the synthesis of cyclopentenoisoquinoline derivatives designed to hydrogen bond specifically to guanine cytosine pairs across the double helix of DNA; (b) the design and synthesis of a new "K cat" inhibitor of dihydroorotate dehydrogenase; and (c) the synthesis of 5-ethynyluracil and its ribo- and deoxyribonucleosides.

SOUTHERN RESEARCH INSTITUTE (N01-CM4-3756)

The objective of this multifaceted contract is to apply fundamental biological principles to developing new and improved *in vivo* and *in vitro* laboratory models to aid the Division of Cancer Treatment in the discovery of more effective anticancer therapies, individual drugs, combinations of drugs, combined treatment modalities, and the optimal conditions for their use. Success of the effort rests on the ability of the Contractor to respond to the Division of Cancer Treatment's needs utilizing test systems of established value, but to a larger extent, to comprehend program needs relative to the development of new and improved biological testing systems; and to respond by conceiving, developing, and using such systems. Tasks conducted may be categorized broadly as (1) service type testing in response to instructions from the National Cancer Institute and in accord with established National Cancer Institute drug screening protocols, (2) detailed studies for which experimental approaches are indicated in more or less detail by the National Cancer Institute, (3) developmental studies indicated as desirable by the National Cancer Institute, but for which methodologies and approaches are expected to be formulated and tested by the Contractor, and (4) exploratory basic studies aimed at the realization of the Division of Cancer Treatment objectives.

SOUTHERN RESEARCH INSTITUTE (N01-CM4-3762)

The chemistry program is based on biological and biochemical rationales, and is integrated with the screening, therapeutic, and biochemical activities at the Institute. The research encompasses the broad categories of both the antimetabolites and the chemically reactive types. The areas of investigation include: deaminase inhibitors, arabinonucleosides, 2'-deoxyribonucleosides, carbocyclic analogs of nucleosides, analogs of adenosylmethionine, nitrosoureido derivatives of nucleosides; folic acid antagonists, and is directed towards the synthesis of: analogs with better transport properties, inhibitors of enzymes other than dihydrofolic reductase, triazenes, hydrazines, and other related compounds. The work on nitrosoureas and cytoxan metabolites is being de-emphasized.

SOUTHERN RESEARCH INSTITUTE (N01-CM4-3780)

The purpose of this contract is to obtain basic quantitative pharmacologic information in animals which will indicate the requirements of drug dosage and scheduling necessary to give maximum tumor cell kill while minimizing toxicity to the host's normal cells. This involves the development and application of highly sensitive analytical methodology to measure drugs and their metabolites in body fluids and tissues and the estimation of half-lives and cumulative Cxt values in animals and man. During the past year studies have involved L-PAM, indicine-N-oxide, asaley, deoxycoformycin, phosphoramide mustard, piperazinedione and prednisone.

SOUTHERN RESEARCH INSTITUTE (N01-CM4-3784)

The major objective of this contract is to develop basic biochemical knowledge useful in the design of new drugs or in the more effective use of known agents. To accomplish this aim, projects are underway on (1) determining if new antitumor drugs have biochemical activities similar to those of clinically established drugs, (2) providing clear leads to biologically significant biochemical properties of new antitumor drugs, (3) evaluating the molecular mechanisms of action of new antitumor drugs, (4) developing systems to allow selection of structural analogs of clinically established antitumor drugs which have more desirable biochemical properties, and (5) developing biochemical rationales for the combined use of clinically established antitumor drugs.

SOUTHERN RESEARCH INSTITUTE (N01-CM5-7000)

The principal objective of this contract is to compare the relative toxicity and therapeutic activities of cancer chemotherapeutic agents alone and in combination when different dosage regimens are used in rodent tumor systems and in non-human primates or dogs. These studies are intended to serve as a basis for the design of a combination drug regimen acceptable for clinical trial. The combination of L-phenylalanine mustard and 5-fluorouracil is currently under study.

SOUTHWEST FOUNDATION FOR RESEARCH AND EDUCATION (N01-CM6-7075)

Synthetic materials of too small a quantity for *in vivo* testing, crude natural products, and fractions of crude natural products that have shown efficacy in the *in vivo* Developmental Therapeutics Program tumor systems are tested *in vitro* at this laboratory. Ths KB tumor system as well as both L1210 leukemia and P388 lymphoid leukemias are employed. Developmental Therapeutics Program protocols are followed and research and development are conducted at the request of the Project Officer. *In vitro* testing is conducted at a 5,000 9KB test equivalent level.

SPARTAN RESEARCH ANIMALS, INC. (N01-CM5-0582) (FORMERLY NIH-75-C-0582)

This procurement contract provides for the supply of 156,000 CD2F1 (BALB/c female x DBA/2 male) hybrid mice for the Developmental Therapeutics Program compound evaluation studies. Breeding animals originate in genetic centers.

ST. LOUIS UNIVERSITY SCHOOL OF MEDICINE (N01-CM5-7020)

The clinical data from brain tumor patients provided under this contract are used to evaluate the efficacy of therapy in both Phase II and Phase III clinical trials. Single agent treatment as well as combined modalities therapy are used as detailed in the protocols of the Brain Tumor Study Group. The Phase III trials evaluate chemotherapy, radiation therapy and combinations thereof (multimodality therapy) following definitive surgery in the treatment of malignant gliomas. New chemotherapeutic agents are also being tested in Phase II trials in order to make the selection of chemotherapeutic agents for future Phase III trials of brain tumor patients more meaningful. The acquisition of patients into these prospective, controlled, randomized studies permits a comparative analysis of the clinical course of the patient and the toxicity of the various therapeutic modalities utilized as well as insight into the biological characteristics of the disease entity being investigated.

STANFORD RESEARCH INSTITUTE (N01-CM3-3723)

This contract is concerned with quality control studies on compounds prepared for preclinical toxicology and clinical trial. The Contractor evaluates bulk chemicals and formulation drugs as to their identity, assay and purity, to provide assurance of high quality and the necessary analytical data for the Food and Drug Administration. This area of work provides elemental analysis, as well as the more sophisticated techniques such as chromatography (paper, thin layer, liquid and gas), absorption spectroscopy (ultraviolet, visible and infrared), nuclear magnetic resonance, and other physical-chemical procedures appropriate for the situation. In some cases, methods of assay must be developed for new structures in order to maintain adequate surveillance on the quality of all drugs used in clinical trials.

STANFORD RESEARCH INSTITUTE (NO1-CM3-3742)

Stanford Research Institute is one of the three major contractors devoted to the synthesis of new chemical structures. The research program encompasses the areas of nucleosides, antimetabolites, antibiotics, alkaloids, and others as necessary. Synthetic effort has continued on the analogs of adriamycin and daunomycin (about 40-50% effort), benzo[i]phenanthridine and ellipticine and intercalators, mesoionic 1,2,3,4-oxatriazoles, analogs of glyoxylic acid tosylhydrazones, piperazinediones, and homomethotrexate. The projected new areas of synthesis are jacaranone analogs, N-(O-nitrobenzenesulfonyl) amino acids, and alanosine analogs. About 10% of the effort is devoted to the resynthesis of active compounds.

STANFORD RESEARCH INSTITUTE (NO1-CM6-3776) (FORMERLY NO1-CM1-2162)

This service preparative contract provides for the synthesis of radiolabeled chemicals and drugs for use in preclinical pharmacologic and clinical studies. Many of the materials prepared are not available from commercial sources and are synthesized. All materials, whether prepared by the contract group or acquired from other sources, are analyzed for purity and identity by radioautography assay, etc. This contract also provides storage facilities for labeled materials and distributes such as directed by the National Cancer Institute staff.

STANFORD UNIVERSITY (NO1-CP6-5825)

As part of a multi-institutional cooperative effort, the effectiveness of routine nutritional practice will be compared with that of total parenteral nutrition via central-line hyperalimentation. Fifteen pediatric patients with malignant tumors of the abdomen and/or pelvis requiring radiotherapy and intensive courses of multiple chemotherapeutic agents will be studied. Comparisons will be made with respect to: (a) tolerance to therapeutic schedule, (b) therapeutic response to combined chemotherapy and radiotherapy, (c) effectiveness of having the bowel "at rest" with respect to effects of therapy on gastrointestinal function, (d) nutritional status of the patient, and (e) complications during therapy. It is hypothesized that maintaining good nutritional status through total parenteral nutrition during combined radiotherapy to the abdomen and intensive chemotherapy, will result in fewer complications, decreased hospitalization time, and better therapeutic response. This hypothesis will be tested by a prospective randomized study comparing the patients maintained by total parenteral nutrition with those on routine dietary intake.

STANFORD UNIVERSITY, BOARD OF TRUSTEES (NO1-CM6-7072)

This contract represents a consortium of clinical centers studying the classification of non-Hodgkin's lymphoma. Because of the plethora of pathologic classifications in this area, the objectives of this collaborative effort are to collect and analyze retrospectively over 1000 cases of non-Hodgkin's lymphoma and correlate the clinical features (and lymphoid markers where available) with various pathologic classifications, seeking the major histologic determinants of clinical behavior. These objectives should be met in a two-year contract period.

STANFORD UNIVERSITY, BOARD OF TRUSTEES (N01-CM6-7111)

This contract represents a consortium of clinical centers studying the classification of non-Hodgkin's lymphoma. Because of the plethora of pathologic classifications in this area, the objectives of this collaborative effort are to collect and analyze retrospectively over 1000 cases of non-Hodgkin's lymphoma and correlate the clinical features (and lymphoid markers where available) with various pathologic classifications, seeking the major histologic determinants of clinical behavior. These objectives should be met in a two-year contract period.

STARKS ASSOCIATES, INC. (N01-CM2-3203)

This service preparative contract supplies the Drug Development Branch with compounds required for drug evaluation studies, with 50% of the effort being devoted to the production of clinical materials. Preparations vary in quantity from 5 grams to multikilograms. The compounds prepared are not readily available in the quantities required on the open market or from the original supplier.

STARKS ASSOCIATES, INC. (N01-CM5-3745)

The Starks Associates, Inc. contract supports the Drug Synthesis and Chemistry Branch's efforts in the acquisition of chemicals and drugs for evaluation in the primary screen. The Contractor is required to: (1) provide liaison services to develop new sources of material and to encourage potential suppliers to participate in the program through voluntary contribution of samples; (2) collect samples; (3) provide support services in the processing of compounds; (4) aid in the management of computer-generated reports relating to compound acquisition; and (5) assist in the development of methods and guidelines for selecting materials for the screen.

STARKS ASSOCIATES, INC. (N01-CM5-3769)

This service contract is for the resynthesis of compounds not readily available from commercial sources or from the original supplier in the amounts needed. The compounds prepared are for the completion of definitive testing or for clinical or preclinical evaluation. About 25% of the effort of this contract is devoted to the preparation of large quantities of materials. Many of the preparations involve multistep sequences.

STEHLIN FOUNDATION FOR CANCER RESEARCH (N01-CM6-7073)

This project provides the needed resource to study the biological growth characteristics, transplantability, genotypic and phenotypic constancy, and response to selected antitumor drugs of human tumor xenografts grown in athymic ("nude") mice. Its major objective is to recommend to the Division of Cancer Treatment human tumor xenograft lines to be used for screening in other projects; that is, those lines which are included in the current DCT panel of tumors. In addition, the Contractor conducts basic studies leading to the development of precise protocols for screening against xenografts including parameters and criteria of drug activity.

TACONIC FARMS, INC. (N01-CM5-0597) (FORMERLY NIH-75-C-597)

This contract furnishes approximately 260,000 B6D2F1 (C57BL/6 female x DBA/2 male) hybrid mice for compound evaluation studies. Breeding animals are furnished from genetic centers and/or rodent production colonies.

TENNESSEE, UNIVERSITY OF (N01-CM0-2084)

The objectives of this contract mainly include formulation development studies and small-batch production of oral, solid dosage forms. The Contractor also has the capabilities to prepare parenteral dosage forms in pilot-batch lots. This contract terminated October 1976.

TENNESSEE, UNIVERSITY OF (N01-CM5-7021)

The clinical data from brain tumor patients provided under this contract are used to evaluate the efficacy of therapy in both Phase II and Phase III clinical trials. Single agent treatment as well as combined modalities therapy are used as detailed in the protocols of the Brain Tumor Study Group. The Phase III trials evaluate chemotherapy, radiation therapy and combinations thereof (multimodality therapy) following definitive surgery in the treatment of malignant gliomas. New chemotherapeutic agents are also being tested in Phase II trials in order to make the selection of chemotherapeutic agents for future Phase III trials of brain tumor patients more meaningful. The acquisition of patients into these prospective, controlled, randomized studies permits a comparative analysis of the clinical course of the patient and the toxicity of the various therapeutic modalities utilized as well as insight into the biological characteristics of the disease entity being investigated.

TEXAS INBRED MICE COMPANY (N01-CM5-3816)

This genetic production center supplies a variety of inbred and hybrid rodents for tumor transplantation, for hybrid production, and for compound evaluation studies. Animals are supplied from a colony of 11 strains of rodents.

TEXAS, UNIVERSITY OF (N01-CM5-3767)

Sublines of murine neuroblastoma and astrocytoma cells (in culture) will be developed to be resistant to antitumor agents of interest to the Division of Cancer Treatment. These sublines will be examined with respect to mechanism of resistance and pattern of cross-resistance to other antineoplastic agents.

TEXAS, UNIVERSITY OF (N01-CM5-3832)

This contract is designed to investigate the role of protected environments and prophylactic antibiotics as adjuvants to the remission induction chemotherapy of non-Hodgkin's lymphoma, metastatic soft-tissue sarcomas, and breast cancer in adults.

TEXAS, UNIVERSITY OF (M.D. ANDERSON HOSPITAL AND TUMOR INSTITUTE) (N01-CM3-3710)

Provides 80 patients (previously untreated) with advanced ovarian cancer to study new agents. Work to date has shown hexamethylmelamine to be an active agent in ovarian cancer and possibly synergistic with alkylating agents.

TEXAS, UNIVERSITY OF (M.D. ANDERSON HOSPITAL AND TUMOR INSTITUTE) (N01-CM3-3737)

This contract has been designed to conduct eradicative clinical trials of patients with bronchogenic carcinoma, explore new and promising avenues of therapy for lung cancer, and devise and implement therapeutic protocols that will lead to a systematic and progressive understanding of the disease and promote its control. During the past contract year new drugs and new drug combinations have been explored in patients with distant metastases.

TEXAS, UNIVERSITY OF (M.D. ANDERSON HOSPITAL AND TUMOR INSTITUTE) (N01-CM5-3773)

The purpose of this contract is to carry out studies on the pharmacology and physiological disposition of new antitumor agents under development by the National Cancer Institute. During the current year, studies have been initiated on ftorafur, thiadiazole, the tricyclic nucleoside (NSC-154020), arabinosyl adenine, arabinosyl-AMP, and the adenosine deaminase inhibitors 2'-deoxycoformycin and erythro-9-(2-hydroxy-3-nonyl) adenine (EHNA). Studies have also continued on the effects of bacterial immunoadjuvants on hepatic enzymes involved in antitumor drug metabolism. Studies under this contract are carried out in experimental animals and in cell culture lines, but are closely interrelated with Phase I clinical trials and clinical pharmacologic studies conducted at the M.D. Anderson Hospital and Tumor Institute and elsewhere.

TEXAS, UNIVERSITY OF (M.D. ANDERSON HOSPITAL AND TUMOR INSTITUTE) (N01-CM7-7149)

A member of the Lung Cancer Study Group: A collaborative group of six (6) institutions which are studying early non-oat cell lung cancer in three (3) protocols. Resectable Stage I tumors are randomized (double blind trial) to intrapleural BCG + INH x12 weeks versus placebo intrapleural BCG + placebo INH x12 weeks. Resectable Stage II and III epidermoid carcinomas are randomized to observation versus radiation to tumor bed and mediastinum versus radiation as above + levamisole. Stage II and III adenocarcinomas and large cell carcinomas are randomized to a three-drug combination of cytoxan, adriamycin, and cis-platinum versus intrapleural BCG + levamisole. An attempt will be made to correlate disease course with certain immune parameters before, during and after surgery.

TEXAS, UNIVERSITY OF, SYSTEM CANCER CENTER (M.D. ANDERSON HOSPITAL AND TUMOR INSTITUTE) (N01-CM4-3786)

The major emphasis of this project is to provide evaluation of the effects of new antitumor drugs on DNA biosynthesis or function, RNA synthesis, processing or function (ribosomal, messenger and transfer RNA) and incorporation into RNA or DNA.

TEXAS, UNIVERSITY OF, SYSTEM CANCER CENTER (M.D. ANDERSON HOSPITAL AND TUMOR INSTITUTE) (N01-CM4-3801)

This contract is designed to conduct Phase I clinical studies with new anticancer drugs sponsored by the Division of Cancer Treatment, National Cancer Institute or to conduct Phase I clinical studies with new combinations or regimens mutually agreed upon. Phase I studies have been conducted on anguidine (NSC-141537), neocarzinostatin (NSC-157365), maytansine (NSC-153858), deazauridine (NSC-126849), gallium nitrate (NSC-15200), hycanthone (NSC-142982) and dichloroallyl-lawsone (NSC-126771) during the past year.

TEXAS, UNIVERSITY OF, SYSTEM CANCER CENTER (M.D. ANDERSON HOSPITAL AND TUMOR INSTITUTE) (N01-CM5-7042)

The Contractor conducts Phase II/III studies in patients with solid disseminated tumors. The tumors to be included are limited to lung, breast, melanoma, soft tissue and bone sarcoma. A minimum of 400 patients a year is studied, with no less than 25 patients in any tumor type. These patients are treated intensively with chemotherapy either alone or in combination with radiotherapy, immunotherapy or surgery in protocols agreed upon by the Project Officer and Principal Investigator. Studies in nutritional support for patients undergoing systemic antineoplastic chemotherapy are also conducted under this contract. Major efforts in melanoma and in breast cancer have contributed to better regimens in the advanced stages of these tumors.

TEXAS, UNIVERSITY OF, SYSTEM CANCER CENTER (M.D. ANDERSON HOSPITAL AND TUMOR INSTITUTE) (N01-CM6-7026)

Ovarian Cancer Study Group Pathology Reference Center: A special task contract to review surgical and postmortem specimens submitted by the Ovarian Cancer Study Group.

TEXAS, UNIVERSITY OF, SYSTEM CANCER CENTER (M.D. ANDERSON HOSPITAL AND TUMOR INSTITUTE) (N01-CM6-7116)

A collaborative group whose main thrust is to study various post-surgical adjuvants in early epithelial tumors of the ovary. A joint protocol for good risk Stage I (i.e., FIGO Stage IAi and IBi) compares observation to L-PAM post-operatively. A secondary study involves a histo-pathologic study of the incidence of unsuspected pelvic and/or paraortic node metastases in this patient population. A further joint protocol for other early disease (i.e., FIGO Stage IAii, IBii, IC, and II) separates patients according to presence or absence of macroscopic residual disease post-operatively. Patients with macroscopic residual disease are randomized to L-PAM versus L-PAM + pelvic radiotherapy. Patients without macroscopic residual disease are randomized to L-PAM versus intraperitoneal instillation of 15 mc of radioactive chromic phosphate.

TEXAS, UNIVERSITY OF, SYSTEM CANCER CENTER (M.D. ANDERSON HOSPITAL AND TUMOR INSTITUTE) (N01-CM7-7153)

Lung Cancer Study Group Pathology Reference Center: A special task contract to review cytology, surgical biopsies, and autopsy material provided by the Lung Cancer Study Group.

TEXAS, UNIVERSITY OF, SYSTEM CANCER CENTER (M.D. ANDERSON HOSPITAL AND TUMOR INSTITUTE) (N01-CP6-5794)

As part of a multi-institutional cooperative effort, the effectiveness of routine nutritional practice will be compared with that of total parenteral nutrition via central-line hyperalimentation. Forty-four pediatric patients with metastatic bone disease requiring radiotherapy and intensive courses of multiple chemotherapeutic agents will be studied. Comparisons will be made with respect to: (a) tolerance to therapeutic schedule, (b) therapeutic response to combined chemotherapy and radiotherapy, (c) nutritional status of the patient, and (d) complications during therapy. It is hypothesized that maintaining good nutritional status through total parenteral nutrition during combined radiotherapy to the abdomen and intensive chemotherapy, will result in fewer complications, decreased hospitalization time, and better therapeutic response. This hypothesis will be tested by a prospective randomized study comparing the patients maintained by total parenteral nutrition with those on routine dietary intake.

TULANE UNIVERSITY (N01-CM5-3815)

This primary genetic center serves to establish foundation colonies of caesarian-derived inbred rats (strains M520, OM/N, Fisher 344, Buffalo, W/L, ALB, ACI and other inbred strains as required). Its objectives are to improve the quality and maintain the genetic characteristics of the strains for program activities. Foundation colonies are propagated in germ-free isolators, and small-scale production colonies are housed in bio-containment environments.

UPJOHN COMPANY (N01-CM4-3753)

This contract has as its primary purpose the studies on the physiological disposition, mechanism of action and biochemical characterization, cell cycle kinetics and combination interactions of selected antitumor agents. Of particular interest during the forthcoming year will be studies on 7-O-methylnogarol and AT-125 in mice. This contract will terminate December 1977.

UPJOHN COMPANY (N01-CM7-7100)

This contract has for its primary objective the research and development of potentially useful antineoplastic agents from fungi, bacteria and actinomycetes, fermentation beers, including isolation, purification, characterization, and production.

The Upjohn Company has played a leading role in the development and exploitation of cell culture methods, both for assays on fractionation samples and for primary screening. Leads developed from this screen are being tested in-house in the leukemia *in vivo* screen and actives are given top priority for chemical fractionation.

Major efforts in this contract are also devoted to biotransformation of known Upjohn antibiotics and phases of toxicological and pharmacological studies within the capability of the Contractor and requested by the National Cancer Institute.

UTAH, UNIVERSITY OF (N01-CM4-3806)

This Contractor is synthesizing fraudulent purines selenium and tricyclic nucleosides as potential antineoplastic agents. The thrust of the current work is the synthesis of nucleosides of greater specificity which are resistant to enzymatic degradation. The areas of investigation include: (1) analogs of tubercidin, sangivamycin, and toyocamycin, (2) derivatives of formycin, (3) pyrazolopyridine nucleosides, (4) imidazopyridine and triazolopyridine nucleosides, and (5) imidazole and benzimidazole nucleosides. In addition to biological evaluation at NCI, this Contractor also has a collaborative effort with Prof. Robert E. Parks, University of Rhode Island, for biochemical evaluation of the materials synthesized.

UTAH, UNIVERSITY OF (NO1-CM5-3827)

The purpose of this project is to determine the pathologic effects of the combination of radiation and adriamycin therapy using the New Zealand white rabbit as a model. It has been well-documented that ionizing radiation can produce cardiac damage in both humans and in the New Zealand white rabbit. Similarly, adriamycin has been shown to produce cardiomyopathies in both humans and the New Zealand white rabbit. With the increased use of adriamycin in treating a variety of human malignancies, particularly when combined with radiation, the question is raised of the possibility of enhanced cardiac damage in radiation or drug dose ranges which have been previously considered to be safe. This contract will terminate September 1977.

VALUE ENGINEERING COMPANY (NO1-CM3-3706)

Data processing services are provided by this contract to the Developmental Therapeutics Program. One area of emphasis is a total redesign and implementation of the Plant Header System. This is an offline file containing plant names, areas of collection and other data important to the Natural Products Branch. The second area of emphasis is the design and implementation of the Terminal Handler System. By means of inputting screening data directly via cathode ray terminal from the large *in vivo* screening laboratories, about one to two months delay in computer processing time could be saved. This contract is to be terminated in August 1977.

VALUE ENGINEERING COMPANY (NO1-CM5-3821)

This contract provides for (1) programming support for the Drug Distribution and Inventory System (DDIS) and (2) technical support for maintaining associated data files. These computer files serve as a record of all drugs sent from the National Cancer Institute to clinical investigators and include all pertinent drug, investigator and protocol information. Manual verification of much of the data on the Clinical Drug Request necessitated the need of a computer file of this information due to the significant increase in the number of Clinical Drug Requests. By using these computer files and displaying the data on cathode ray terminals, the data technicians minimize the time required to process drug requests and use the data on the actual file thus improving accuracy and reporting.

VALUE ENGINEERING COMPANY (NO1-CM6-7086)

For ease of contract management, the previous Value Engineering Company contract (NO1-CM3-3706) was given a new contract number at the time of renegotiation. Data processing services are provided by this contract to the Developmental Therapeutics Program. The main areas of emphasis are: (1) reducing and disseminating information developed in the screening program of the Drug Evaluation Branch; (2) documenting all programs and contractor's procedures for data handling and running computer programs; (3) maintaining the computer programs so that they are able to run in the Division of Computer Research and Technology environment; (4) reducing and disseminating information on the receipt of compounds including those of particular interest to the Division of Cancer Treatment program; (5) expanding the present system to incorporate secondary testing not previously stored on the master files; (6) redesigning the existing system and developing new formats and methods of data collection; (7) providing instructions for screening laboratories and suppliers of materials relating to collection and dissemination of data; (8) providing output for statistical evaluation of test systems and evaluation of test system parameters; and (9) participating in scientific meetings.

VANDERBILT UNIVERSITY MEDICAL CENTER (N01-CM7-7122)

A member of the Lung Cancer Study Group: A collaborative group of six (6) institutions which are studying early non-oat cell lung cancer in three (3) protocols. Resectable Stage I tumors are randomized (double blind trial) to intrapleural BCG + INH x12 weeks versus placebo intrapleural BCG + placebo INH x12 weeks. Resectable Stage II and III epidermoid carcinoma is randomized to observation versus radiation to tumor bed and mediastinum versus radiation as above + levamisole. Stage II and III adenocarcinoma and large cell carcinoma are randomized to a three drug combination of cytoxan, adriamycin, and cis-platinum versus intrapleural BCG + levamisole. An attempt will be made to correlate disease course with certain immune parameters before, during and after surgery.

VERMONT, UNIVERSITY OF, COLLEGE OF MEDICINE (N01-CM3-3716)

This contract provides for the extension of the Computerized Problem-Oriented Medical Information System to the Division of Cancer Treatment by integrating structured input ("cancer displays") into the system, thereby coupling learning with doing for all personnel who care for individual cancer patients entered on problem-specific plans (research protocols).

VETERANS ADMINISTRATION (Y01-CM7-0106) (FORMERLY Y01-CM4-0004)

Collaborative Program for Clinical Investigation of Anti-cancer Therapies by the Veterans Administration Cooperative Groups. Tasks performed by the Lung Cancer Chemotherapy Group, the Surgical Adjuvant Chemotherapy Group, and the Brain Tumor Study Group are: (1) pharmacology and introduction of new anticancer agents into clinical use; (2) improve use of chemotherapy agents of established value; (3) improve treatment of patients with lung cancer; (4) study benefits of surgical adjuvant chemotherapy; and (5) evaluate treatment of patients with inoperable lung cancer.

VETERANS ADMINISTRATION (Y01-CM7-0107)

This is an Interagency Agreement with the Veterans Administration Hospital to support the Medical Oncology Branch whose primary objective is to study lung cancer and other adult tumors in the Veterans Administration Hospital. This contract was originally set up as a means of expanding our intramural capability to study common human tumors. Lung cancer, being the number one cancer killer in men, was selected as a prime target for clinical trials with cancer chemotherapy. The purvue of contract is being shifted from the Cancer Therapy Evaluation Program (formerly part of the VA lung cooperative studies) to the Clinical Oncology Program which will only support the Medical Oncology Branch at the VA Hospital. This Branch is under the direction of Dr. John Minna. In the last two years, the Branch has been completely reorganized. Clinical trials and related laboratory investigations have been designed in a variety of tumors seen commonly at the Veterans Hospital. The Veterans Hospital provides a remarkable resource of patient material for studies which could not be undertaken at the Clinical Center. This medical oncology resource allows an expansion of our intramural drug-testing effort.

VETERANS ADMINISTRATION HOSPITAL (SOUTH CAROLINA) (Y01-CM6-0102)

The principal objective of this contract is to synthesize transition metal coordination compounds with antitumor activity, high aqueous solubility and reduced renal toxicity. Current effort is focused on several approaches to obtain the desired potential drugs, including conversion of platinum complexes to a higher oxidation state to increase the water solubility and the synthesis of complexes with "onium" ligands to change the site of excretion of unused drug. Two of the synthesized compounds are of high interest as potential replacements of the clinically used diammine-dichloro-platinum in the treatment of cancer.

VIRGINIA, UNIVERSITY OF (N01-CM6-7002) (FORMERLY N01-CM1-2099)

The major objective of this contract is the fractionation of confirmed active plants in order to isolate in a pure state and identify the antitumor active components. Plant materials used in this work are obtained, for the most part, through the United States Department of Agriculture.

WALTER REED ARMY MEDICAL CENTER (Y01-CM4-0005)

This Interagency Agreement is in support of Walter Reed Army Medical Center (WRAMC) participation in Cancer and Leukemia Group B as a full group member. WRAMC has participated actively in all the protocols of the CALGB entering 66 patients in studies during 1976. The Principal Investigator and staff have attended all meetings and are developing multimodality functions in medical oncology, pediatrics, pathology and radiotherapy. WRAMC staff generated eight new protocols during 1976 and 180 new patients were entered on WRAMC protocols. The Principal Investigator has been involved in planning of the Head and Neck Study Program and the Testicular Study Program. He is also a medical oncologist in the Gynecology Oncology Group.

WARF INSTITUTE, INC. (N01-CM4-3707)

This represents a service contract to prepare extracts of plant materials for screening by the screening laboratories. The great majority of plant extracts screened by the National Cancer Institute originate with this contract. The majority of the Contractor's plants are obtained through a Transfer of Funds Agreement between the National Cancer Institute and the United States Department of Agriculture. Special studies of methodology of plant extraction are conducted in order to maximize the yield of confirmed active plants.

WARF INSTITUTE, INC. (N01-CM5-3770)

This is a service contract for *in vivo* screening of potential anticancer agents. Currently, testing is being conducted at a level of approximately 50,000 L1210-equivalent tests per year. Agents tested include new crude plant extracts, fractionations of previously confirmed natural products, new synthetic materials, and follow-up testing of active materials. Testing is conducted in mice in accordance with National Cancer Institute protocols. The P388 lymphocytic leukemia is employed as the model for the initial testing of all materials. Additional models now in use under this contract include a mouse colon tumor (colon 26), a mouse lung tumor (Lewis lung carcinoma implanted intravenously), the B16 melanoma, and the L1210 leukemia. Schedule dependency studies are conducted for materials of potential clinical interest. Methodology and/or research and development, as well as special research projects, are scheduled as the need arises and are directed by the Project Officer.

WASHINGTON UNIVERSITY (N01-HB6-2970)

This contract is concerned with clinical studies to determine the efficacy of granulocyte transfusions and in what patient population they are useful.

WASHINGTON UNIVERSITY SCHOOL OF MEDICINE (N01-CM4-3731)

The cytocidal effects of single drugs on normal hematopoietic stem cells and malignant cells (L1210, AKR, B-16 melanoma, and Lewis lung) are quantitated by using a spleen colony or lung colony assay. Drug pairs are then evaluated for their effects on tumor and host cell survival. From these data principles for drug-pair sequence and dose intervals may be disclosed.

WAYNE STATE UNIVERSITY (N01-CM6-7105)

The Contractor conducts Phase II/III studies in patients with solid disseminated tumors. The tumors to be included are limited to lung, breast, melanoma, soft tissue and bone sarcoma. A minimum of 400 patients a year are studied, with no less than 25 patients in any tumor type. These patients are treated intensively with chemotherapy either alone or in combination with radiotherapy, immunotherapy or surgery in protocols agreed upon by the Project Officer and Principal Investigator. Studies in nutritional support for patients undergoing systemic antineoplastic chemotherapy are also conducted under this contract. Major efforts in melanoma and in breast cancer have contributed to better regimens in the advanced stages of these tumors.

WISCONSIN, UNIVERSITY OF (N01-CM1-2275)

The principal objective of this contract is to study the mechanism of replication of genetic information, contained by RNA-tumor viruses, as well as the mechanism of integration of this information into the host chromosome. To accomplish this objective, studies are in progress with purified viral enzymes in order to understand clearly the enzymic reactions involved. The results on each isolated reaction will be assessed with relation to the physiology and life cycle of the intact virus. The DNA and RNA enzymes have been implicated in the etiology of human cancer. An understanding of the properties and mechanism of these enzymes is, therefore, fundamental for the eventual solution of this problem. This contract terminated April 1977.

WISCONSIN, UNIVERSITY OF (N01-CM6-7076)

This contract provides rapid cell culture cytotoxicity assays as an aid in isolation and purification of potential antitumor agents of microbiologic origin. Standardized assays are performed in accordance with procedures described by Developmental Therapeutics Program protocols employing KB, L1210, and P388 cell lines. Occasional methodology studies may be carried out in consultation with the Project Officer.

YALE UNIVERSITY SCHOOL OF MEDICINE (N01-CM4-3797)

This contract is designed to evaluate a combined modality protocol employing radiotherapy and chemotherapy in the treatment of locally unresectable carcinoma of the pancreas.

YALE UNIVERSITY SCHOOL OF MEDICINE (N01-CM5-3824)

The purpose of this contract is to mount a chemotherapeutic attack, by means of new agents designed and prepared under this contract, on the neoplastic cell membrane, particularly on the synthesis of its glycoprotein components,

YALE UNIVERSITY SCHOOL OF MEDICINE (N01-CM5-3824) (CONTINUED)

aimed at either inhibition of membrane synthesis or alteration of functional aspects of the membrane such as antigenicity, cellular adhesiveness, and/or mobility, cell cycle determinants, or the state of cellular differentiation. As new agents are synthesized, their impact on a suitable number of the above mentioned biological effects is determined.

YALE UNIVERSITY SCHOOL OF MEDICINE (N01-CM5-3843)

This contract is designed to explore in prospective randomized, controlled protocol studies (1) the value of adjuvant chemotherapy following a curative gastric resection, and (2) adjuvant therapy of locally advanced, surgically incurable gastric carcinoma comparing single agent chemotherapy versus combination chemotherapy versus single agent plus radiotherapy.

YALE UNIVERSITY SCHOOL OF MEDICINE (N01-CM5-7029)

The objective of this contract originally was to produce new anticancer agents based on their ability to intercalate and bind to nucleic acids. This would interfere with strand separation, which might be expected to result in suppressed or faulty replication of DNA or RNA. Evaluation of the agents synthesized was first by physical measurements of binding strength, melting temperatures, then by studies at the molecular level of effects on the replication and processing of various nucleic acids, and finally by antitumor effects *in vitro* and *in vivo*. Correlation between *in vivo* antitumor effectiveness and the *in vitro* and molecular-level effects is sought.

YALE UNIVERSITY SCHOOL OF MEDICINE (N01-CM6-7068) (FORMERLY N01-CM3-3711)

The objective of this contract is to provide *in vitro* screening systems to select promising anticancer agents of the antifolate type. The compounds are assayed for inhibition of partially purified dihydrofolate reductase from L1210 leukemia cells, from human leukemias and normal red blood cells. Special preclinical pharmacologic studies are carried out with selected agents, and appropriate data are gathered from patients receiving this type of drug. This is the first year of a continuation, as a result of competition, of contract N01-CM3-3711.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 06401-05 OD

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Data Processing Systems for Scientific and Management Information

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	M. Gordon	Systems Analyst	OD DCT NCI
OTHER:	L. Greene	Computer Specialist	OD DCT NCI

COOPERATING UNITS (if any)
Data Management Branch, Division of Computer Research and Technology
National Cancer Program, Management Information System

LAB/BRANCH

SECTION
Office of the Director, Division of Cancer Treatment

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.0	1.5	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The Cancer Treatment Program is stratified for purposes of monitoring, analyzing and planning the allocation of resources. A stratification form is used by DCT staff to collect, aggregate, and tabulate monthly budgetary information. This information is then evaluated periodically to determine the adequacy of funding for each R&D line item.

The raw data for this DCT budget management system is obtained by the automation of contract data input via RCB, NCI; direct operations data input via DFM and the NCP-MIS; and cooperative group grants information via DCRRC, NCI. The stratification forms have been designed to capture Disease- and Modality- oriented budget information, which is then applied to the financial data. Differentiation between "Research" and "Support" is also indicated.

With the assistance of DMB, DCRT; a mailing label system is being developed. This system will enable us to consolidate the diverse mailing lists now in use at DCT and is similar to the system already in operation at DRG, NIH.

PHS-6040
(Rev. 10-76)

Project Description

Objectives: 1. To establish a complete DCT budget management system by the automation of contract data input via RCB, NCI; direct operations data input via DFM and the NCP-MIS; and cooperative group grants information via DCRRC, NCI.

2. Revision of the DCT Stratification to provide comprehensive Disease-Oriented and Modality-Oriented budget information.

3. Establishment of a microfiche information system for archival and distribution purposes.

4. Development of a mailing label system.

Methods Employed: 1. Through the use of DMB, DCRT program generation software, a single file containing all DCT contracts (commitments, Schedules A&B), direct operations data, and grants data is updated monthly at the computer terminal. This update is via the DMB, DCRT UPDATE and EDIT system; the data is entered into the WYLBUR Reformat Generator (WYLGEN) for conversion to the appropriate transaction format. This update is designed for use by nonprogrammers. All entries in the file are classified with respect to the DCT Stratification. Differentiation between "Research" and "Support" is also indicated.

2. New Stratification forms have been designed to capture additional Disease- and Modality-oriented budget information. Concurrently, programs have been written by DMB, DCRT to reinitiate the DCT data file by use of this new information. Contractural information procurement continues as before, with the TRW-generated linkage to the RCB, NCI contract management system for monthly updates. Cooperative Group grants information is now obtained from DCRRC, NCI via WYLBUR and from DRG via the IMPAC system.

3. Monthly reports are now distributed and stored on microfiche.

4. With the cooperation of the Special Assistant to the Director, DCT, NCI; a consolidated DCT mailing label file is maintained and updated when necessary.

Major Findings: 1. Programs have been written by DMB, DCRT to generate reports by various combinations of the DCT Stratification elements applied to contract, direct operations, and grants data as well as by DCT organization. In particular, several new reports are now available for classification of the DCT budget by the "new" DCT Stratification. Emphasis is placed on Disease- and Modality-oriented summary reports. DCT is now producing reports that were previously done manually by the Administrative Officers with the use of DFM reports. DCRRC, NCI provides DCT with grants information in the form of a WYLBUR data set.

2. Regeneration of the DCT data file has allowed for the incorporation of many new variables, which pertain to the Cooperative Group grants that have been transferred from DCRRC. Accordingly, many new budget reports may soon be generated upon request.

3. An effort will be made to incorporate a "Keyword" potential in the DCT data file similar to that of Cancerline.

4. The availability of contract and DCRT account data has enabled this office to formulate the annual ADP plan requested by the OD, NCI. Statistics pertaining to the Data Processing expenditures of DCT are now available via the "new" DCT Stratification as applied to the DCT budget.

Significance to the Program of the Institute: The computer system that has been developed with the assistance of DMB, DCRT is utilized to monitor and assess budget information related to segments of the DCT program and to more specific project areas both in the intramural areas and in the contract program. The reports generated by this system lead to a detailed description of the DCT research program.

Proposed Course: Possible modifications of the new stratification form to allow for the receipt of treatment data from other divisions of NCI is contemplated. These modifications will, in turn, require another large scale revision of the information system with the subsequent generation of new reports by DMB, DCRT. Programs will also be written to generate NCP approach category 6 reports via fixed relationships with the stratification.

Additional reports will also be prepared using the AUTOTAB system to show budget balance type information. This system, which is a software package leased by DCRT from CAPEX Corporation, facilitates the preparation of financial reports. The system was greatly enhanced this past year by the introduction of the external file capability. It is this capability, which will make it possible to link AUTOTAB programs to intermediate summary data generated by the DMB, DCRT programs for Stratification-oriented reports.

Automation of the retrieval of DCRT account data from DCRT tapes in the form required by the annual ADP plans will be investigated. This data combined with DCT stratification data will be used to generate reports of DCT data processing expenditures.

An automated mailing label system for all of DCT will lighten the secretarial workload and lead to the elimination of duplicate efforts within DCT. The "CANCER TREATMENT REPORTS" mailing lists are being used as a basis for the initiation of a larger DCT database. The two other major sources of labels for the initiation of the database are the Cooperative Group files and the lists of Project Officers and Principle Investigators for DCT contracts.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 07025-03 OD

PERIOD COVERED
October 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Biomedical Engineering for Intramural Research, DCT

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: W. Penland, Biomedical Engineering Project Officer, OD, DCT, NCI

OTHER: DCT NCI V. DeVita, B. Lewis, R. Atkinson (OD); J. Zeigler, J. Bull (CO); A. Deisseroth, P. Pizzo (PO); R. Magin, C. Meyers, S. Shackney (LCHPH); R. Bender (MB); J. Minna, P. Bunn, M. Cohen (VAMO); S. Schimpff, P. Weirnik (BCRC); P. Sarin, C. Saxinger, R. Gallagher (LTCB); V. Bono(ID); L. Kirkland (RO); S. Rosenburg, P. Sugarbaker, M. Brennan (SURG); others. Arthur D. Little, Inc. A. Wechsler, A. Burg, J. Ketteringham, S. Battista (Program/projects); C. Kensler and staff (Mgt. Review); J. Lyddy, D. Lindsay, E. Cook, R. Liss, K. Wiig, A. Neilson, others (Projects). Los Alamos Scientific Laboratories (ERDA) P. Mullaney, G. Salzman (PI's). Oak Ridge National Laboratories (ERDA) J. Breillatt, C. Remenyik (PI's). U.S. Naval Weapons Center M. Benton, D. Sticht (PI's). Artek Systems Corporation C. Leonhardt (PI).

COOPERATING UNITS (if any)
RTI/NASA Technology Utilization, R. Scearce; LTD, NHLI, P. Carmesi; NIH Clinical Center, NR, G. Johnston; Becton-Dickinson Research Center, K. Abel, Block Engineering, Inc., S. Shapiro.

LAB/BRANCH
Office of the Director, Division of Cancer Treatment

SECTION
Biomedical Engineering Office

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
14.0	13.0 (12 contracts)	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline k

Sustained collaborative biomedical engineering support, mainly of an exploratory/developmental nature, has addressed the feasibility of engineering concepts and methods; provided novel instrument research and development; and furnished analytical support and technical consultation for clinical and pre-clinical investigations of the DCT Program during this period.

Broad engineering resources are being focused upon the characterization and implementation of useful engineering projects for a diversity of intramural needs in: tumor cell kinetics, hyperthermia studies, specialized clinical instrumentation and products, tumor cell biology, blood cell procurement and preservation, pharmacology studies and radiation therapy.

Project Description

General Objectives/Scope

1. To provide broadly based and specialized biomedical engineering (BME) resources to the research program of the Division of Cancer Treatment, NCI.

2. To identify and characterize problems amenable to BME solutions and to conduct BME research and development efforts responsive to the diverse research programs, projects and tasks of individual investigators of the Division of Cancer Treatment and collaborating groups.

3. To develop and refine approaches for the effective management of broad BME resources through the contract mechanism.

Clinical Instrumentation and Projects

The expertise and time required for current colonoscopy limits wide application of diagnostic procedures that could dramatically improve the management of colonic disease. Promising conceptual approaches for rapid colonoscope advancement, combined with means for external localization of the colonoscope tip have been developed and early prototype models are being evaluated (Sugarbaker, NCI; Lyddy, Battista, ADL). The initial prototype employs sophisticated inflatable cuffs which allow the colonoscope tip to advance itself. Localization of the colonoscope tip via fiberoptic light, thermal and magnetic techniques is being explored. Ultimately, an automated and computerized manually directed system with sigmoid, splenic flexure, tranverse colon, and decending colon modes, may be possible.

An experimental fiberoptic UV transmitting bundle was develped this period to expand diagnostic capabilities of the bronchoscope (Cohen, NCI; Lyddy, ADL). This device will be used to irradiate lung tumors with UV light. Tumors absorbing hematoporphyrin, administered intravenously, are expected to fluoresce bright red and be capable of bronchoscope observation. Previous experiments and preliminary *in vivo* investigations at Mayo Clinic have motivated this development. The most significant technical problem involved the fabrication of a small quartz fiberoptic bundle.

A new semi-conductor crystal, cadminum telluride, not available commercially, was incorporated into a 5 mm broncoscope (Lyddy, ADL) to detect gamma emitting radioisotopes differentially localized in malignant lung tissue. Technical difficulties involved specific packaging and finding a low noise cable (Swedish). Initial clinical performance is planned (Cohen, NCI).

The quantitation of growth in colonies of marrow granulocyte procursors for studies of abnormalities in white cell proliferation (Bull, NCI), has been supported for two years by prototype devices developed by the U.S. Naval Weapons Center in collaboration with NHLI (Carmesi). Techniques for the automatic measurement of spatial frequency components of bone marrow colonies in a gelatin field are being exploited. A second instrument with extended range and improved operating features was sought and achieved this period. Technical documents and information from the DCT staff has resulted in development of potentially marketable units through Becton-Dickinson & Co. and Artek Systems, Inc.

A very small inflatable broncoscope cuff, not commerically available, and a series of small diameter broncoscope needles for aspiration of lung fluids were provided Dr. Cohen through the ADL contract this period.

A bacterial filtration system as well as a series of line connector designs were proposed and evaluated for safe long term intravenous alimentation studies this period (Brennan, NCI; Battista, Lyddy, ADL).

In response to a requirement to measure blood flow in peripheral tissues, a capacitance plethysmograph similar to that of the Joslin Research Clinic (T. Aoki) is being developed and tested (Brennan, NCI; Lyddy, ADL).

Modified versions of the NCI biological isolation garment are being considered for a high degree of patient protection and broader clinical use. Commerical headpieces, filter masks, backpack units and garments have been obtained for study (Pizzo, Schimpff, NCI; others outside NCI).

Cell Kinetic Instrumentation and Studies

A lengthy competative technical evaluation was accomplished this period for the development of a TV based cell scanning system to complement flow microfluorimetry (FMF) studies of tumor cell classification and related interests (Shackney, Bunn, others, NCI). This system semi-automatically obtains the integrated optical density of the cell nucleus through silouetting techniques and counts autoradiographic grains within the cell nucleus or cyto-cytoplasmic areas on a cell-by-cell basis. Data display can take multiple forms. This system represents the first economical semi-automatic image processing unit for accelerating cell kinetic studies of the NCI and collaborative groups. It will become commercially available in early 1978.

Techniques of multiparameter flow cytophotometric characterization for counting and classifying cell populations in the blood of leukemia patients were explored (Shapiro, Block Engr.). Peripheral blood samples from patients with leukemia, provided by Dr. Wiernik of BCRC, were examined to obtain a comparison between cell morphology obtained by human observers and multiparameter flow cytophotometric analysis. The measurements provided information on the distribution of parameters related to cell size, nucleic acid content, specific granule content, cytoplasmic granularity and surface roughness. The multiparameter measurement technique is more likely to be able to detect residual blast populations than most other techniques and shows promise as a rapid and precise method for characterization and classification of blood cell populations in patients with leukemia.

The feasibility of using multiparameter flow cytophotometric instrument of Block Engineering to identify and count bone marrow and megakaryocytes, and to determine the size and nuceic acid content distributions of these cells was explored (Bunn, NCI; Shapiro, Block Engr.). Although the feasibility of multiparmater flow cytophotometry was demonstrated, other more available and less complex instruments are probably equally suitable for the task of megakaryocyte counting, sizing, and DNA content analysis.

Flow microfluorometry (FMF) instrumentation and biological support from the Los Alamos Laboratories during this period covered; (a) continued development of the potential sensing technique for cell volume analysis, (b) continued improvement in advanced data processing software, (c) on- site technical support for correcting FMF malfunctions in flow chambers, cell sorting hardware and electronics and for laboratory transfer and re-establishment of the DCT FMF system, (d) study of the interaction between various fixatives and stains in support of refined measurement techniques for a variety of model tumor cell lines of DCT interest and (e) publication and reporting of technical progress and results. A renewal agreement which continues similar activities was approved this period.

Several exploratory techniques for measuring DNA synthesis rates rapidly in flow microfluorometry (FMF) systems were presented by Dr. Howard Shapiro and reviewed by the DCT staff this period.

Radiation Diagnosis and Therapy

A study of the feasibility of computers to plan and control radiation therapy procedures ("dynamic" radiation therapy), is in progress (Kirkland, NCI; Lindsay, ADL). Since completely automated devices for such therapy are not a practical reality, the feasibility of novel and useful software and electro-mechanical hardware will be studied to determine whether a dynamic system (a) can administer increased radiation doses to the tumor volume with consistant reduction of exposure to adjacent normal tissues and (b) can treat the types and incidence of malignancies where local control is not (or seldom) achieved with conventional methods. Should the utility of a dynamic system appear favorable, the development of performance specifications for competative procurement will be considered.

In previous whole-body radioautography studies (Bender, NCI; Liss, ADL) the localization of α-methyl dopa in the rodent pancreas suggested its use in clinical gamma camera monitoring of normal and neoplastic pancreatic tissue. During this period, the feasibility of incorporating the gamma-emitting radioisope of Selenium into the α-methyl dopa molecule was exaimed through several steps of chemical synthesis. Difficulty with obtaining high yields in a critical synthesis step, prevented completion of the project. Dramatic improvements through the combined use of CT scans and ultrasound imaging of pancreas tissue also diminished the potential utility of this project.

Imaging problems frequently caused by surgical clips in brain scanning procedures of computerized tomography systems have been investigated (Rosenburg, NCI, Doppmann, NIH, Gempel, ADL, Scearce, RTI). Since the metallic composition and motion of the clip appear primarily responsible, the evaluation of titanuim clips and possibly new polymer compositions are being evaluated. Corrective software measures appear impractical.

In response to imaging problems of a neurotoxicity assay using CT scanning (Poplack, NCI; DiChiro, NIH) which require uniform density interface (packing) between the subjects head and scanner components, conceptual approaches involving the remote transport of plastic beads or fluids to inflatable transparent bladders are being investigated (Penland, NCI; Lyddy, ADL).

Blood Cell Procurement/Preservation

The initial goal of the ORNL Project to achieve a high yield rotor (Cytriage) for granulocyte collection via continuous flow centrifugation was exceeded during this period of intensive clinical trials (Deisseroth, Ungerleider, NCI; Breillatt, Remenyik, ORNL). Leukapheresis of human donors with the Cytriage at NCI yielded a mean collection rate of 4 x 10^9 PMN/hr using heparin-ACD anticoagulant. When hydroxyethyl starch was added to the donor blood entering the rotor, the rate doubled to ca. 8 x 10^9 PMN/hr. The projected use of hormone stimulation to double-triple the donor's circulating PMN pool should raise the PMN yield to 2 x 10^{10}/hr at current extracorporeal flow rates of 40-50 ml/min: equivalent to 6 x 10^{10} per 3 hour run. Such yields are currently of great interest in adequately reversing established sepsis. In early Cytriage trials, the plasma/RBC interface position was unstable making it difficult for the automated control to reach and maintain optimum operating conditions. This instability was reduced tenfold by inserting a horizontal septa assembly into the radial flow chamber, which served to distribute the Ekman layers across the axial length of the chamber. The positioning of the interface is the operational key to maximizing PMN collection; but in the early trials, optimum positioning required most of the running time. Now, near-optimum interface position is rapidly and efficiently attained through electronic and logic modifications to the control system whereby the hematocrit in the WBC line is used as a reference for continuing automatic adjustment of the interface signal from the fiber optic sensor. New Cytriage units are being considered for extended trials at DCT and with collaborating institutions.

U.S. Patent, 3,955,755, Closed Continuous-Flow Centrifuge Rotor; Inventors; J. Breillatt, C. Remenyik, K. Sartory, L. Thacker, W. Penland; was assigned to ERDA, May 11, 1976.

Engineering assistance this period for several areas of blood cell procurement and preservation included; acquisition of experimental fibers and design development of potentially new fiber leukapheresis systems; development of gas sterilization techniques for new rotors; acquisition of NASA cryogenic equipment for cell preservation; development of microwave thawing equipment for bagged blood (Georgia Tech, ADL, RTI/NASA).

Hyperthermia Projects

A project plan and technical request for proposal (RFP) was developed this period (Penland, collaborating NCI groups, DCT staff) to investigate, develop, validate and furnish advanced instrumentation to produce controlled local hyperthermia and improved thermal dosimetry anticipated for diverse *in vivo* clinical and pre-clinical investigations. The program provided for radiation oncology groups, among others, to substantially guide engineering hardware development by setting instrumentation criteria and by conducting important in vivo evaluations as key users. CTPS reviews of this program were unfavorable because of competing research priorities and insufficient data to forcast general hyperthermia effectiveness when applied alone or with other treatment modalities.

Engineering support for the Phase I evaluation of hyperthermia and radiation therapy protocols by the RTOG Program was developed and partially implemented during this period. The ADL staff has recommended microwave and thermometry equipment, developed information for equipment procurement and operation and has planned on-site technical assistance to RTOG members regarding equipment use and calibration and definition of data recording methods, etc. (Lewis, Atkinson, Penland, NCI; Johnson, RPMI).

Technical support for the development of a microwave hyperthermia apparatus for mice (Magin, NCI) covered improvements in the microwave network and wave guide applicators (Cook, ADL). A proposed network design was developed and tested satisfactorily. New microwave applicators were obtained and tested at BRH for heating pattern characteristics that appear useful in future applications.

Focused ultrasound heating of deep lying tissues and its potential was examined in the laboratory of P. Lele, MIT, November 18, 1976 (DCT and ADL staffs).

Chemical Pharmacology

Pharmacokinetic studies (R. Bender, NCI) have demonstrated that CEM lymphoblasts rapidly transport and concentrate vincristine. The feasibility of determining binding sites of vincristine in cultured human CEM lymphoblasts at selected growth phases is being determined by radioautography (Liss, ADL). Information is being sought to clarify how cytoplasmic microfilament and membrane binding may interfere with mitosis and cell membrane functions.

Initial electron microscopy support was given by R. Liss, ADL, for the ultrastructural analysis of murine tissues treated to minimize adriamycin cardiotoxicity (Myers, NCI). Support is continuing through another contract.

An optical apparatus for providing appropriate radiation to animal tumors treated with hematoporphyrin (Dougherty, et.al.), was developed and applied in an ADL study (Merker) for Dr. Venditti, DCT.

Tumor Cell Biology

A programmable, automatic apparatus which processes fractionated cell samples through some 30 sequential operations to extract reverse transcriptase over an 8 hour period in a cold room environment was developed and tested successfully this period (Saxinger, Coy, NCI; Lyddy, ADL). Such instrumentation shows potential for a variety of preparative biochemical techniques requiring repetative labor saving operations. Applications to the DCT Biomarkers Program is being investigated.

Mathematical Modeling

ADL (Richards) is considering methods of improving a structure activity model (Hodes, DCT) for preselection of drug compounds for prescreening. A project to increase the separation of active from inactive compounds and to identify new compounds is being sought.

The discrete time kinetic model (Woo, NCI; Wiig, ADL), which is capable of generating cell age distribution and predicting the cell size and DNA content distributions, was extended this period to establish a quantitative relationship between tumor cell number and biological marker concentrations. The growth kinetics of Chinese hamster cells was quantitatively related to their polyamine content, and the total polyamine concentration during synchronization was calculated and related to the total cell number. Publication of this work is in press (Clinical Chemistry).

Antibiotic Administration

Poor out-patient compliance with antibiotic regimens used at BCRC and elsewhere is traceable to unpalative flavor properties of antibiotics whose successful administration often requires more than hospital staff support and patient education. Initial diagnostic flavor analyses and the development of suitable "white taste" flavors for Vancomycin HCL 500, Gentamycin sulfate 200, and for Nystatin suspensions have been initiated as part of a four-phase project to systematically improve flavors of these compounds (Schimpff and BCRC staff; Neilson and ADL staff).

Major Consultations

DCT investigators were provided consultation on the following subjects by the Arthur D. Little staff during this period: Definition, Planning and Evaluation of Individual Biomedical Engineering Projects for DCT (Penland); Investigation of Real Time Determination of Viral Antigenicity with the Block Virometer (Galliher); Establishing a Clinical Record System Project (Minna); Review of Sensitive Fluorescent Detection Instrumentation for Intracellular Tags (Saxinger); Instrumentation Approaches to Counting EB Virus Transformed Cells (Pizzo); Production of a Biomaterial Effective in the Specific Removal of Antigen/Antibody Complex (Zeigler); Production of Antisera Specific for Bromodeoxyuridine and for Guanine in DNA (FMF Synthesis Rate Determination) (Bono); Simultaneous Total DNA and Bromodeoxyuridine Labelling Determination in Cultured Cells by Flow Cytometry (Bono); Fabrication of a Broad Band NMR Spectrometer (Sarin); Head Positioning Device for CT Scans (Penland); Special Food Services for Protective Environment Study (Pizzo); Calibrated Slides for Radioautographic Tests (Shackney); Analysis of Surgical Clip Imaging Problems (Rosenburg); Early Conceptual Approaches to Colonoscope Advancement (Sugarbaker); Hyperalimentation Apparatus Improvement (Brennan); TV Cell Scanning Instrumentation Design Requirements (Penland); Engineering Consultation and Project Support for DCT

Biomarker Program (Penland); Experimental Megakaryocyte Separation Techniques (Bunn); Staff Evaluation of NCI/ADL Biomedical Engineering Projects, 1973-77 (Penland; Quantitative Laboratory Measures of Proliferation in the Non-Hodgkin's Lymphomas (Shackney).

Significance

This biomedical engineering activity relates to a number of exploratory research areas in the Division of Cancer Treatment that can potentially benefit from bioengineering approaches. This activity is successfully focusing diverse technical resources to quickly and usefully respond to DCT laboratory and clinical research interests. It is broadening the scope and data base of the biological and medical scientist to achieve specific research goals. It thereby increases the probability that new engineering methodologies and instrumentation will contribute as in the past, to short and long range goals of the Division of Cancer Treatment.

Proposed Course

Next year's biomedical engineering resource program will have several major thrusts: (1) To provide clinicians at DCT (including BCRC and VA) with useful prototype biomedical instruments to assist their diagnosis and monitoring of therapy; for example, through an expanded effort with colonoscope development, UV and optical broncoscopy instruments, drug delivery devices, etc. (2) To respond to investigators at other collaborative institutions and other divisions of NCI where the subjects of interest and resultant development efforts have a common basis; for example, hyperthermia support, drug delivery devices, instrumentation for cell kinetic studies, etc. (3) Attempt to support important new areas such as the biomarker effort (FCRC), drug screening program (DS&CB), etc. (4) To systematically explore potential projects with all new DCT staff and those not yet contacted for opportunities in project development.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01-CM-09050-02) Z01-CM-09051-02) OD Z01-CM-09052-02)

PERIOD COVERED
October 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Physical Science Collaborative Research

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

E. R. Atkinson, Physical Science Project Officer, NCI/DCT/OD

COOPERATING UNITS (if any) Nat'l Aeronautics & Space Adm., Food & Drug Adm., Nat'l Bureau of Standards, Dept. of Defense, Presidential Office of Telecommunication Policy, Energy Research & Development Adm., Environmental Protection Agency, Occupational Safety & Health Administration.

LAB/BRANCH
Physical Science Project Office

SECTION
N/A

INSTITUTE AND LOCATION
Division of Cancer Treatment, National Cancer Institute, Bethesda, Maryland

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
5	4	1

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline k

Physical science collaboration is being conducted in: automated tissue culture colony counting; millimeter wave spectroscopy of tumor cells; whole body hyperthermia patient treatment; whole body hyperthermia animal studies; toxicity of chemotherapeutic agents at elevated temperatures; non-thermal effects of microwaves; precision measurement of size of biological objects; mass spectroscopy of biopolymers and unnatural isotopes; nuclear magnetic resonance of membrane specific proteins; human thermoregulation; receptor site tertiary structure by electron spin resonance; dielectric properties of normal and tumor tissues; pion production at low energies in the laboratory frame; tissue sensitizers for ionizing radiation; microwave imaging techniques; ultrasonic temperature measurement; liquid crystal effects in biological materials; optical and viscometric techniques for temperature measurement; actin and myosin mechanics.

REPORT OF THE ASSISTANT DIRECTOR FOR INTERNATIONAL TREATMENT RESEARCH
OFFICE OF THE DIRECTOR

The international activities of the Division of Cancer Treatment are developing rapidly and an Assistant Director's Office for International Treatment Research was established in 1976 within the Office of the Director, Division of Cancer Treatment, to coordinate these activities. In the office are A. Goldin, Assistant Director for International Treatment Research, I. Kline, Expert Consultant to the Assistant Director, O. Yoder, NCI Collaborative Office Representative, A. Santoni, Visiting Fellow and B. Nolte, Secretary. The international activities encompass the interests of the various program areas of the Division of Cancer Treatment and their relationships to international treatment research. This involves the development and continuing conduct of collaborative preclinical and clinical programs, exchange of personnel, procurement and exchange of synthetic agents and natural products for screening and development, and the identification of new compounds of potential clinical interest.

The activities of the international program of the Division of Cancer Treatment are dovetailed closely with the Associate Director's Office for International Affairs, NCI.

There are strong relationships with international organizations such as the International Union Against Cancer (UICC), the World Health Organization (WHO), the European Organization for Research and Treatment of Cancer (EORTC), The International Agency for Research on Cancer (IARC) in Lyon, and the Pan American Health Organization (PAHO).

The operation of the Cancer Chemotherapy Research Collaborative Office at the Institut Jules Bordet in Brussels, with Dr. O. Yoder serving as the NCI Division of Cancer Treatment Officer continues to be highly successful in furthering the overall collaborative program in Europe. The operation of the Collaborative Office, which was established in 1972, encompasses both liaison activities and the conduct of a chemotherapy research laboratory at the Institut Jules Bordet. The Chemotherapy Office continues to play a most important role in collaboration and in development of cancer research and treatment in Europe. The Chemotherapy Office functions in close collaboration with the EORTC providing a most important link between cancer research in Europe and the National Cancer Institute in the United States.

The headquarters of the EORTC is at the Institut Jules Bordet in Brussels with Professor Henri Tagnon as the current President.

The EORTC which has a highly sophisticated organization was instituted in 1962 by the primary cancer research institutions of the Common Market countries and Switzerland. Representatives of Great Britain joined in 1966 and representatives of Austria in 1970. Since then participation has been extended to representatives of Eastern Europe. Hungary has associate membership status and the COMECON (Socialist) countries have official representatives in the Scientific Council of the EORTC. The EORTC has in its Administrative Board and

Scientific Council twenty-five members representing ten countries in Europe and fifteen experimental and clinical fields. The Scientific Council plans and coordinates the activites within the organization. Dr. Yoder serves as a de facto member of the Scientific Council and as a member of the EORTC Screening and Pharmacology Group which reviews candidate drugs for clinical trials within the EORTC. The Administrative Board of the Council is responsible for administrative and financial matters. Clinical and laboratory cooperative studies are conducted by thirty-four separate units which include clinical cooperative groups, project groups, clubs, working parties and task forces. The activities of the project groups and cooperative groups cover a wide area ranging from organic synthesis and preclinical activities through treatment of various types of human neoplasia. The EORTC has formal recognition by WHO as one of its regional institutions. THe EORTC publishes the European Journal of Cancer and the EORTC Newsletter. A European Foundation was created last year, under the auspices of the EORTC and it is functioning well in providing the EORTC with Financial backing. Mr. Grierson, A London Banker serves as the manager of the Foundation. The Council of the Foundation consists of prominent personalities in various member countries who are committed to fund collection. It is planned that the EORTC will be essentially self supporting within about three years.

The Data and Coordinating Center is proving its value particularly in view of the expanded activities of the EORTC. The Data Center is functioning with grant support of DCT, NCI which will continue until the end of 1978. Dr. M. Staquet is the Director of the Data and Coordinating Center (and also is EORTC Coordinator). He has a supporting staff of fifteen people including: statisticians, computer analysts, programmers, data managers, secretaries, and data clerks. The Data Center provides supporting services for the various EORTC groups involved in clinical trials. There are forty-two ongoing clinical trials and the data center has over 4000 cases in its active file involving the participation of the major cooperative groups within the EORTC. It has a protocol review committee with representatives from the U.S. as well as from Europe which reviews all new protocols prior to initiation of a clinical trial within the EORTC. The Breast Cancer Task Force was created in September, 1975. Publications of the EORTC appear regularly in the medical literature.

In the last year, approximately 5000 new materials were received from European sources for antitumor screening. More than 100 compounds have shown confirmed activity in at least one tumor system, under NCI guidlines. An additional 50 compounds have shown preliminary activity and are awaiting confirmation. A significant number of new active compounds has undergone secondary investigation, and two of these (the nitrosourea derivative RFCNU, and Formylleurosine) are currently undergoing Phase I clinical trial in Europe. The current interest in hexamethylmelamine and its analog pentamethylmelamine stems from observations in England that these drugs are highly active against human tumor xenografts in athymic mice.

Through the collaborative Chemotherapy Office a close working relationship is maintained with the Mario Negri Institute and the National Tumor Institute in Milan,the Institute of Cancer Research in London, the University of Leeds, the Laboratories of the Medical Research Council in Dublin, the TNO Institut in Rijswijk, the Institute of Cellular Pathology in Louvain, the Hopital St. Pierre in Brussels, and with various other laboratories in both Western and Eastern Europe.

During the past year, with the collaboration of the Chemotherapy Office in Brussels, a Foreign Exchange Nurse Visitor Program was implemented. One nurse has now arrived at the NCI from the Royal Marsden Hospital in London and will stay for a period of approximately six months. The arrival of a second foreign exchange nurse from Utrecht in the Netherlands is being finalized and the tentative date of arrival is August. It is planned for a third foreign exchange nurse fom Europe to arrive in the fall of 1977 or early winter of 1978. It is also planned to send a first Clinical Center NCI nurse to the Royal Marsden Hospital in London in early fall, 1977. Overall,it is planned to have a sustained and augmented effort in the foreign exchange visitor program, with extension to various countries in Europe. There will be approximately six European nurse exchanges per year for six month periods. The objectives of the Nurse Exchange program encompass exchange of information, techniques and latest developments in nursing research and collaborative participation in research programs in the Institutions visited.

The rapid development of the EORTC and its importance as a focal center in Europe was contributed to in significant measure by NCI moral and financial support over the last few years. The NCI Collaborative Office in Brussels has played a major role in this regard, both materially and in terms of focusing public attention in Europe on the multi-national aspects and importance of cancer research.

In addition to the overall international activities in Europe there are a series of bilateral programs, and in these, close contact is maintained with the Office of International Affairs, NCI.

The French-American Agreement for Clinical Trials and Treatment Research is relatively new and it involves the Division of Cancer Treatment, NCI and the Institut National de la Sante et de la Recherche, Medicale (INSERM).
Dr. Vincent DeVita is the chairman of the U.S. Group and Dr. Georges Mathe is the chairman of the French Group.

FRENCH - AMERICAN AGREEMENT
FOR CLINICAL TRIALS AND TREATMENT RESEARCH COMMITTEE

FRENCH		AMERICAN	
Mathé	Hopitalier Paul Brousse, Villejuif	DeVita	NCI
Israel	Hopital Franco - Musulman, Paris	Muggia	NCI
Boiron	Hopital Saint - Louis, Paris	Frei	Farber Cancer Institute
Jacquillat	Hopital Saint - Louis, Paris	Mihich	Roswell Park
Hayat	Hopitalier Paul Brousse, Villejuif	Chabner	NCI
Calle	Foundation Curie, Paris	Goldin	NCI
Lagarde	Universite Bordeaux, Bordeaux	Schein	Georgetown
Meyer	INSERM, Marseille	Gutterman	M.D. Anderson
		Jaffe	Farber Cancer Institute
		Macdonald	Georgetown

The first meeting of the Joint Committee was held September 25, 1976 at the INSERM Center in Paris. Subsequently the American Committee members met in Bethesda December 10, 1976. The second meeting of the Joint Committee was held February 25-26, 1977 in Bethesda. At the latter meeting a number of collaborative projects were agreed upon as follows:

		AMERICAN	FRENCH
1.	Pharmacology and Clinical Trials	Schein	Mathé
	(Phase I and II) of New Nitrosourea Analogs	Muggia	
	RCFNU - Possible reduced immunosupressive properties	Goldin	
	RPFNU - Possible reduced immunosuppressive properties		
	Chlorozotocin - Possible reduced myelosuppressive properties		
	Other Analogs Antitumor activity, Pharmacology, Clinical Activity		

2.	Osteogenic Sarcoma - High Dose MTX - Radiation 1. Intensive treatment for limb preservation. Surgical resection. Intraarterial adriamycin and 4 weekly courses V - MTX - CF. 2. Post amputation treatment similar to 1. 3. Treatment of inoperable tumors - Eight weekly courses V-MTX-CF plus Radiation 6-8 weeks. 4. Treatment of metastasis V - MTX - CF 4-8 weekly courses. If complete remission - V - MTX - CF and ADR at 3 weekly intervals for total duration of 18 months.	Frei Jaffe	Calle Schweisguth Tubiana
3.	Plasmapheresis - removal of blocking substances		
	Phase I and II study to evaluate role of plasmapheresis in management of patients with advanced "untreatable" cancer. Lymphocytes of cancer patients cytotoxic for their tumors In Vitro (Hellstroms) Serum from cancer patient results in specific blocking of cell mediated cytotoxicity. Specific blocking factor in serum either tumor specific antigen or tumor antigen - antibody complex (Sjogren).	Schein Macdonald	Israel Edelstein
4.	Clinical Biochemical-Pharmacology A. Pharmacokinetic studies		
	MTX 1. Comparison fluorometric and enzymatic or competitive binding methods	Mihich Chabner	Meyer

2. Effects of hepatitis and renal dysfunction on drug elimination

3. Drug interactions which could affect renal clearance (platinum compounds, antibiotics, salicylates)

5FU

1. Setting up high pressure liquid chromatography as assay method and comparison gas chromatography and mass spectrometry.

2. Influence of routes and schedules

B. Pharmacological studies at Villejuif To be determined according to project developed	Mihich Chabner	Mathe Bohuon and Paoletti
5. Phase II trials with anticancer drugs		
A. Platinum compounds (testicular cancer after relapse other chemotherapies, leukemias, lymphomas, melanomas, breast cancer, ovarian and lung cancer).	Muggia	Hayat
B. Anthracyclines Preclinical and clinical - new analogs	Muggia Goldin	Jacquillat
C. Others for later consideration		
Diglycoaldehyde	Muggia	Boiron
Acronycine	Muggia	Boiron
Maytansine	Muggia	Boiron

6.	G.I. Cancer Trials		
	A-1. Adjuvant chemotherapy - Gastric Cancer 5FU - ADR - Mit. C (FAM)	Schein Macdonald	Boiron
	A-2. Treatment advanced gastric cancer Ftorafur - ADR - Mit. C (FAM II) (FURAM)		
	B. Combination chemotherapy - Pancreatic cancer 5FU - ADR - Mit. C (FAM)	Schein Macdonald Muggia	Boiron Lagarde
7.	Clinical investigations systemic irradiation versus Metastatic occult (micrometastases) lung cancer Large single fractional doses (ca 800 rad) delivered as:	Muggia Rubin	Israel Mathé Tubiana

A. Hemi-body (or whole organ)

B. Large segmental strips:
Plus the following options

Radiosensitizers
Hyperthermia
Optimally timed chemotherapy
Immunopotentiation
Bone marrow Autotransplantation

8. Phase I and II Immunotherapy

Phase I Studies — Gutterman — Israel

A. Intralesional immunotherapy, mutant endotoxin plus P3 (glycolipid mycobacterial fraction) plus cell wall skeleton

B. Intravenous immunotherapy. Methanol extracted residue of BCG (MER)

Phase II Studies on Chemoimmunotherapy

A. Breast Cancer — Gutterman — Israel
   1. 5FU - Adriamycin - Cyclophosphamide (FAC) plus C. parvum
   2. 5FU - Adriamycin - Cyclophosphamide (FAC) plus pseudomonas vaccine

B. Melanoma — Gutterman — Jacquillat
   1. Adjuvant therapy recurrent malignant melanoma with DTIC and Actinomycin D ± intravenous C. parvum

9. Tamoxifen in advanced breast cancer — Schein, Macdonald — Lagarde, Boiron, Calle

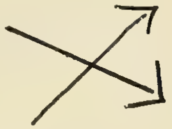
(Tamoxifen and antiestrogen competes with estrogen for binding to cytosol estrogen receptor proteins)

Tamoxifen has undergone Phase II trials in selected patients with breast cancer. 40% objective response (Morgan, Schein)

Investigations

A. Pre-menopausal female
   Oophorectomy - (relapse patients treated with tamoxifen)
   vs
   Oophorectomy plus tamoxifen

B. Intramenopausal female
   Fluoxymesterone (androgen)
   vs
   Tamoxifen



cross over to determine cross resistance

C. Postmenopausal female
   Diethylstilbesterol
   vs
   Tamoxifen

Responders (stable or regressing) held for determination "rebound regression"

Progressing - cross over to determine X resistance

There are three additional cooperative projects of interest for later activation:

1. Effect parenteral hyperalimentation on immune response of patients with solid tumors	Muggia Schein	Meyer
2. Hyperthermia	DeVita	Lagarde
3. Studies with liposome encapsulated drugs		

The French-American Agreement for Clinical Trials and Treatment Research has gathered considerable momentum in a short time and there is strong potential for highly significant progress. This is particularly so since the French and U. S. investigators have paired off in teams on specific problems.

A program is under active development involving both clinical and preclinical activities in Latin America. The status of this US-PAHO-Latin America Program in which Dr. Muggia is the primary DCT clinical representative and which is being conducted in coordination with the International Cancer Research Data Bank, Office of International Affairs, NCI, is as follows:

The scope of the program encompasses.

1. Strengthening of interactions with identified cancer centers
2. Collabortive protocol studies and antitumor drug support
3. Exchange of investigators
4. Joint workshops
5. Information and education exchange
6. Acquisition of candidate antitumor materials
7. Collaboration on selected preclinical research problems.

Collaborative protocols are in planning stages for the following disease entities:

1. Osteosarcoma
2. Melanoma
3. Breast Cancer
4. Stomach Cancer
5. Cervical Cancer
6. Testicular Cancer
7. Esophageal Cancer

Institutions and investigators have been paired off for purposes of working together, and the planning of the program has gotten off to a very good start.

The listing of collaborating institutions and investigators is as follows:

	U.S.	LATIN AMERICAN
1.	Roswell Park Memorial Institute Edward Henderson	Bogota, Colombia Instituto Nacional De Cancerologia Julio Ospina
2.	U. Texas M. D. Anderson Hospital Gerald Bodey	Rio De Janeiro, Brazil Instituto Nacional De Cancer Adayr E. deAraujo
3.	Sloan Kettering - Memorial Vermont Regional Cancer Center Joseph F. Burchenal Irwin Krakoff	Sao Paulo, Brazil Instituto Central-Hospital A.C. Camargo Fundacion Antonio Prudenta F. C. Gentil
4.	Comprehensive Cancer Center for State of Florida Francisco Tejada	Lima, Peru Instituto Nacional De Enfermedades Neoplasticas Eduardo Caceres
5.	University Wisconsin Hospitals Guillermo Ramirez	Santiago, Chile Departmento de Enfermedades Hematologicas Oncologicas y Banco de Sangre Univ. Cat. de Chile Juan Arraztoa
6.	Division of Cancer Treatment, National Cancer Institute Division of Oncology Georgetown Univ. Hospital J. Zielger F. Muggia A. Goldin P. Schein	Argentina Instituto Angel H. Roffo A. Olivari Servicio de Oncologia del Hospital Military Central R. Esteves Servicio de Oncologia de Hospital Rawsin Koliren

Servicio de Hematologia
de la Acadamia Nacional de Medicina
    S. Pavlovsky
Possibly also:
Municipal College for Oncology
    J. Schavelxon

There have already been a number of visits by U.S. Investigators to their South American Counterparts.

There are bilateral programs in the Middle-East and Africa.

1. A bilateral program is under development between the US and the Republic of Egypt. Dr. J. Ziegler is the primary clinical DCT representative in this program which is conducted in coordination with the Office of International Affairs, NCI

Cairo Cancer Institute - NCI Collaborative Project
   A. Phase II Studies in "Bilharzial" Bladder Cancer
   B. Collaborative studies Radiosensitizers, immunology, and carcinogenesis may be added as project evolves.

US - NCI	EGYPT
Ziegler	Cairo U. Cancer-Inst.
Anderson	El-Sebai
Chevlen (on site as liaison)	

2. Uganda Cancer Institute Contract -
   Follow up of Burkitt lymphoma and Hodgkins' disease patients treated over past 10 years

US	Uganda
Ziegler	Olweny

		US	
3.	Hepatitis B. vaccine proposal Workshop scheduled to up-date sero-epidemiology and demography hepatitis B virus and hepatoma worldwide Identification of an area where clinical epidemiologist could undertake vaccine trial.	Ziegler - NCI Fogarty NIAID Bureau of Biologics	
		US	Israel
4.	Hebrew University - Immuno-Adjuvant effects of MER in murine systems and in clinical trials in lymphoma/leukemia. (Available data - no therapeutic benefit - may be terminated)	Ziegler	Weiss
		U.S. - Israel Binational Foundation	
		U.S.	Israel
5.	Antitumor screening and evaluation of compounds obtained from plants in Israel	Goldin	Lavie

The bilateral agreement with the USSR, established in 1972, involves various areas of cooperation in medical science and health.

The portion of the US - USSR Joint Agreement pertaining to chemotherapy involves:

Exchange of preclinical and clinical drugs
Screening, pharmacologic and toxicologic assessment of drugs.
Cooperative clinical investigations and clinical information exchange.
Exchange of scientists
Joint publications

In the drug exchange the U.S. has provided the Soviets with a total of fifty-nine drugs, thirty-four of which are in clinical use or trial and twenty-five of which are in preclinical testing. The Soviets in turn have provided the U.S. with ninty-six drugs of which twenty-eight are in clinical use and sixty-eight are in preclinical testing. One of the Soviet drugs is Ftorafur, a slow release form of 5-fluorouracil. It was synthesized in Hiller's Institute of Organic Synthesis in Riga, Latvia and it is currently in clinical investigation in the U.S. Another Soviet compound of interest is the anthracycline, carminomycin, which was isolated in Gauze's Institute of Antibiotics in Moscow. It has about the same order of activity in animal tumor systems as does adriamycin or daunomycin. Interest in carminomycin stems from the observation that it is active on oral administration and that it possibly has diminished cardiotoxicity as compared with Adrimycin or Daunomycin. Additional new Soviet drugs of program interest include Prospidine, Reumycin, Dioxadet, Variamycin, Imidaphen, Fenterin.

Definitive progress was made during the last year on a collaborative monograph entitled "US - USSR Preclinical Antitumor Chemotherapy Test Data and Clinical Correlations". Included in this monograph are the results of antitumor studies of thirty US and thirty USSR drugs in a spectrum of US and USSR tumor systems. The analysis of the collective data is the primary objective of the monograph and the following problems are being addressed:

(a) To compare the data that was obtained employing the same methodological approaches with both common and different tumor model systems.

(b) To conduct an analysis of the experimental data in relation to clinical activity in order to establish the possibility for prospective prediction of antitumor activity of new drugs in the clinic, both for antitumor activity in general and for activity against specific categories of tumor. For this purpose, biological, biochemical and specific mathematical approaches are being employed, taking into account not only the data collected in each country but also the common data.

(c) To employ the analysis for the improvement of screening systems in each country.

Included in the monograph, in addition to the presentation of the test data analysis, will be a listing of the drugs employed, their structures and physico-chemical characteristics and descriptions of the tumor systems. It is considered that the results of this collaborative investigation may point the way to the improvement of screening methodology and to future investigations and approaches for obtaining new effective drugs for the clinic.

Cooperative clinical investigations and clinical information exchange encompass three working groups:

Breast Cancer - Davis
Lung Cancer - Muggia
Ovarian Cancer - Young

Clinical studies with USA anticancer drugs in the USSR may be summarized as follows:

(a) Melanoma	Act D - VCR - Methyl Nitrosourea (MNU) as effective as Act D - VCR - CCNU - DTIC and more effective than ACT D - VCR - CCNU
(b) Lung cancer	Cytoxan and MNU slight superiority over cytoxan and CCNU (not definite)
(c) Breast Cancer	Hexamethlmelamine (USA) and Ftorafur (USSR) individually active
(d) Stomach cancer	CCNU and Ftorafur and Ftorafur alone comparable or superior to CCNU alone, 5FU alone and CCNU and 5FU
(e) GI Cancer	Streptozotocin and Ftorafur slightly better than streptozotocin and 5FU

During the Fourth USA - USSR Annual Cancer Chemotherapy Review in the Soviet Union 5 - 14 April, 1976 meetings were held in the following areas:

1. Conference on ovarian cancer
2. Meeting for "Correlation of Anticancer Drug Screening Systems"
3. Second USA - USSR Conference on "Lung Cancer Treatment"
4. Annual Cancer Chemotherapy Program Review

During the week of May 23-27, 1977 the following USA-USSR Chemotherapy Groups met in the Bethesda area:

1. May 23-25 Working meeting of the preclinical chemotherapy group.
2. May 22-24 Second conference on Lung Cancer Treatment.
3. May 24 - US-USSR Conference on the treatment of breast cancer
4. May 23-24 US - USSR conference on Ovarian Cancer.

Agreements were signed outlining further collaborative studies in the above preclinical and clinical areas.

The U.S. - Japan Cooperative Cancer Research Program was established in May 1974 between the Japanese Society for the Promotion of Science and the National Cancer Institute. The objectives of the program include:

1. Exchange of information
2. Standardization of clinical trials
3. Exchange of criteria and information on new treatments and protocols
4. Setting up of collaborative protocols

Program areas involved in the collaboration include cancer therapy in general, breast cancer, lung cancer, bladder cancer, cancer immunology, metastasis, and radiation. Specific project areas involved in the collaboration include:

A. Gastric Cancer - Diagnosis and Treatment
B. Nitrosourea compounds
C. Bleomycin and derivatives
D. Anthracycline antibiotics

A collaborative gastrointestinal study group program is in progress and has had a number of meetings:

Dec. 1975	NCI DCT Delegation to Japan - Discussion G.I. Cancer
July 1976	Japanese Delegation to Boston - Discussion Gastric Cancer
Sept. 1976	NCI DCT Delegation to Japan - Status nitrosourea analogs Collaborative program gastric cancer in progress- common arm 5FU plus Adriamycin

The Northern California Cancer Program is highly active in this program. There have been a number of joint meetings in the last few years:

Feb. 1975 in Tokyo	A. Joint Seminar "Comparative Study on cytosine arabinoside and cyclocytidine B. Workshop on Phase I Studies
Feb. 1976 in Hawaii	Joint symposium on the status of clinical trials of bleomycin and nitrosoureas
Oct. 1976 in Kyoto	3rd Annual Joint Meeting U.S. - Japan Cooperative Research Program
May 1977 San Francisco	Antitumor antibiotics

There are two important contracts in Japan and it is through these contracts and personal contacts that the NCI program has considerable entree into the various research institutes, Universities and Pharmaceutical Houses in Japan.

1. Japanese Foundation for Cancer Research, Tokyo for the operation of a Collaborative Office for Cancer Therapy Research (Sakurai). The Project Officers on this contract are A. Goldin and F. Muggia. This collaborative Chemotherapy Office is similar to the one at the Jules Bordet Institute in Brussels. It is involved in therapy liaison activities, drug collection for screening, personnnel exchange, information exchange, monitoring of new drugs in clinical trial, drug screening and evaluation and clinical investigations with new drugs. The data collected in the screening programs in both Europe and Asia are entered into the DCT Computer Program.

2. Microbial Chemistry Research Foundation, Tokyo for the study of potential antitumor agents of microbial origin (Umezawa). The NCI Project Officer is J. Douros. It is through this program that the NCI has made available to it, at an early date, the most interesting antibiotics stemming from a highly extensive antibiotics program in Japan.

Another binational cooperative program involves the US and Poland This is a health agreement between the National Cancer Institute in the US and the Institute of Oncology in Poland which was established in 1973. It involves the exchange of information and materials, and exchange of scientists.

An additional binational cooperative program is between the U.S. and the Federal Republic of Germany initiated in May, 1975. This involves the NCI and the Ministry of Research and Technology. It entails exchange of information and scientific personnel, and collaborative program development One Program under development involves collaborative investigations of human tumors growing in athymic mice. This is between the NCI and Professor G. C. Schmidt's laboratory in Essen.

Although there is no formal Medical Agreement with the United Kingdom there are strong preclinical and clinical research affiliations, particularly with the Institute of Cancer Research in London. Contracts in England include:

		US Project Officers
Institute of Cancer Research London (Harrap)	Model System Development and agent collection	Venditti - Wood
Institute of Cancer Research Surrey (Lamerton)	Studies of Animal Tumor cell kinetics	Schackney
University of Leeds Leeds, (Cooper)	Phase II trials in gastrointestinal carcinoma	Muggia

There are strong collaborative preclinical and clinical therapy programs with a number of institutes in Italy and a range of contract activities.

Contracts in Italy		US Project Officers
Farmitalia, Milan (Arcamone)	Design and synthesis of derivatives and analogs of doxorubicin	Wood - Engle

Farmitalia, Milan (Bernardi)	Design and synthesis of aglycones related to doxorubicinone	Wood - Engle
Mario Negri Inst. Pharmacological Research, Milan (Garattini)	Collection and Screening neoplastic agents	Abbott
Mario Negri Inst. Pharmacological Research Milan (Spreafico)	Studies in Immunochemotherapy Research	Goldin
Mario Negri Inst. Pharmacological Research Milan (Garattini)	Relationship of antineoplastic drug kinetics to *in vivo* effects	Zaharko
Instit. Nazionale per lo studio e la cura dei tumori Milan (Veronesi)	Controlled clinical trials for breast and brain tumors	Muggia
Instit. Nazionale per lo Studio e la cura dei tumori Milan (Veronesi)	Coordinating center for clinical study of melanoma	Fisher - Muggia
Instit. Nazionale per lo Studio e la cura dei tumori Milan (Rilke)	Classification of Non-Hodgkins' lymphoma	Berard
Clinica Neurochirurgica dell Universita di Pavia Pavia (Paoletti)	Therapy of patients with brain tumors	Strike - Walker

Additional contract activities include:

Contract in Netherlands		U.S. Project Officer
Radiobiological Institute Organ. for Health Research Rijswik (van Putten)	Studies on the combination of radiation and chemotherapy	Goldin

Contract in Spain		U.S. Project Officers
University of Madrid Madrid (Gosalvez)	Characterization of antitumor drugs. Details of effects on electron transport and energy metabolism of mitochondria	Abraham-Bono

Contracts in Belgium		U.S. Project Officers
Institut Jules Bordet Brussels (Attasi)	Primary and Detailed in vivo screening for anticancer activity	Macdonald - Abbott

It is of interest to list the Foreign contracts by country and the financial expenditures:

	Dollars
Italy	927,300
England	96,800
Japan	113,300
Belgium	357,000
Holland	67,100
Spain	31,000
Uganda	125,000
Israel	107,400
Total	1,824,900

The Foreign Grants (Clinical Cooperative Trials Program) are as follows:

Hopital Saint-Louis, Paris Bernard (44 T)	Acute Leukemia Group B.	Hugh Davis
Hopital Franco-Musulman, Paris Israel (38.4 T)	ECOG	Hugh Davis
U. Pretoria, South Africa Falkson (58 T)	ECOG	Hugh Davis
Jules Bordet, Brussels Tagnon (278 T)	EORTC Coordinating and Data Center	Hugh Davis
Univ. Montreal, Canada Band (31 T)	ECOG	Hugh Davis
Jewish General Hosital, Montreal Margolese (31.5 T)	Primary Breast Group	Hugh Davis
Royal Victoria Hosp., Montreal Shibata (24.9 T)	Primary Breast Group	Hugh Davis
Total 506T		

The total expenditure in the foreign program is modest but the return has been rather great. In this connection it is of interest to list antitumor drugs with established clinical activity that have come from countries abroad.

Drug	Source
Cyclophosphamide	Germany
Busulfan (Myleran)	England
Adriamycin	Italy
Daunomycin	Italy
Mitomycin C	Japan
Bleomycin	Japan
L-Pam (Sarcolysin)	England - U.S.S.R.
5-Azacytidine	Czechoslovakia

It is also of interest to note antitumor drugs from other countries that are in clinical investigation:

DRUG	Source
Ifosphamide	Germany
Dibromomannitol	Hungary
Dibromodulcitol	Hungary
Dianhydrogalactitol	Hungary
Rubidazone	France
ICRF 159	England
RO7-0582 (Radiosensitizer)	England
VP 16 (Podophyllin Derivative)	Switzerland
VM 26 (Podophyllin Derivative)	Switzerland
Ftorafur	Latvia-USSR
Carminomycin	USSR
Cyclocytidine	Japan
Yoshi 864	Japan
Neocarzinostatin	Japan

Visits were made by A. Goldin as follows:

1. In a trip June 21 - July 3, 1976 (with F. Muggia, M. Slavik and O. Yoder) travel was conducted as follows:
   A. Participation in EORTC Immuno-Oncology Program and EORTC Screening and pharmacology group session in Paris and in Villejuif.

   B. Site visit to Jules Bordet Liaison Office and to EORTC Operations Office in Brussels.

   C. Site visit to Dr. L. van Putten's Laboratory at the Institut of the Organisation for Health Research (TNO), Rijswijk, The Netherlands for discussion and exchange of scientific information.

   D. Site visit to Mario Negri Institut; visit to Instituto Nazionale per lo Studio de la Cura di Tumori to meet with Dr. Di Marco and Staff and Dr. Veronesi and staff for preclinical discussions on anthracyclines and overall clinical therapy program and visit with scientists of Farmitalia for further discussions on collaborative studies with anthracycline derivatives. A visit was also conducted to Dr. A. Nicolin at the Pharmacology Department, Milan University School of Medicine for discussion of collaborative studies.

   E. A visit was made to the laboratory of Dr. E. Bonmassar in Perugia, Italy at the Institut of Pharmacology, University of Perugia for discussion of collaborative investigations and for participation in a seminar on preclinical and clinical programs at the National Cancer Institute in Perugia.

2. On September 2 through September 12, 1976 a visit was conducted to the Cancer Research Center in Moscow. We brought 10 U.S. anticancer drugs to the Soviet Union under the U.S.-U.S.S.R. Agreement and in return brought back to the NCI a total of fourteen drugs. During this visit discussions were conducted with Drs. Anatoli Syrkin and Zelina P. Sophina in the review of data and further planning of the

collaborative monograph "Analysis and Correlation of Screening Systems of Anti-Cancer Drugs in the U.S.A. and U.S.S. R."

We participated in a workshop on "Human Tumor Sampling for Biochemical-Pharmacological Studies of Target Determinants of Drug Action" at the Cancer Institute in Moscow under the auspices of the International Union Against Cancer (UICC/CICA). This program was also supported in part under the US-USSR Medical Agreement. An attempt will be made on the basis of this workshop to encourage collaboration on pertinent problems between various laboratories represented at the workshop and also with other laboratories and institutes where there is specific expertise and interest.

A visit to the Institute of Organic Synthesis in Riga served to renew and reinforce the relationship with this Institute. The visit was considered important in view of the death of Professor S. Hiller with whom we had developed a good working relationship particularly with reference to ftorafur which was synthesized in this Institute. The visit was conducted by Drs. Chabner and Goldin. We met with Drs. Lidak and Polis. Visits were also made with Dr. Chipens who is the current Director of the Institute and other members of his staff.

3. On September 23-24, 1976 participated and presented a paper at an "International Symposium on Recent Advances in Cancer Treatment" conducted under the auspices of the European Organization for Research on Treatment of Cancer (EORTC). The symposium was held at the Institut Jules Bordet in Brussels in honor of Dr. Henri Tagnon.

The first Joint Committee Meeting under the French-American Agreement on clinical trials and treatment research was held at the Institut National de la Sante et de la Recherche Medicale (INSERM) on September 25th at the INSERM Center in Paris. Dr. Vincent DeVita served as the Chairman for the meeting which was highly successful in the outlining of specific areas for potential collaboration. A detailed report of the meeting has been written by Dr. Gregory O'Conor.

Participated in the Sir Stanford Cade Memorial Symposium on the Interaction of Radiation and Antitumor Drugs at the Royal Institution in London on September 27-28, 1976. This meeting was notable in that it brought together preclinical and clinical investigators from two disciplines in the treatment of neoplastic disease, namely radiation therapy and chemotherapy. The symposium was particularly timely in view of the strong National Cancer Institute interest in the combined modality therapy of radiation and chemotherapy. The focus at this meeting on the compound RO70582 is notable in view of our current interest in the development of this radiation potentiator for clinical use.

4. On October 11-15, 1976 with O. Yoder participated in a Cancer Chemotherapy course in Wisla, Poland. The course was sponsored by the Polish Oncological Society including the Institute of Oncology and Institute of Immunology and Experimental Therapy; the Commission of Tumor Biology of the Department of Medical Sciences at the Polish Academy of Sciences in Wroclaw; the Center of

7. On April 13-15, 1977 (with O. Yoder) participated in the VII International Symposium on the Biological Characterization of Human Tumors in Budapest, Hungary. The symposium was conducted under the auspices of the Coordinating Committee for Human Tumor Investigations and the Hungarian Cancer Society. It was also sponsored by the International Agency for Research on Cancer (IARC) in Lyon, France. Major subjects included at this symposium were: Molecular basis of viral carcinogenesis; molecular basis of chemical carcinogenesis; hormones and cancer; chemotherapy of solid tumors; breast cancer. In addition there was a series of proffered papers on chemotherapy and chemical carcinogenesis; breast cancer; biochemical guidelines to selective anti-tumor chemotherapy in animals and man. The session on chemotherapy of solid tumors is worthy of note particularly in view of our increasing program emphasis on the utilization of athymic (nude mice) and thymectomized mice for the growth of human tumors, and the application of chemotherapy with individual drugs; polychemotherapy and combined modalities including immunochemotherapy, radiation plus chemotherapy and surgery plus chemotherapy. There is a strong interest abroad and significant contribution in the growth and treatment of human xenografts and this appears to be providing a new dimension in studies on prospective prediction for the clinic for individual drugs, drug combinations and combined modalities.

An opportunity was also afforded to speak with Dr. Janos Berdy in Budapest concerning his classification system for antibiotics. The Berdy classification system has been developed on a collaborative basis between Dr. Janos Berdy and the Director Dr. Tibor Lange of the Research Institute of Pharmaceutical Chemistry in Budapest, the Frederick Cancer Research Center, the National Cancer Institute's Office of International Affairs and its International Research Data Bank and the Division of Cancer Treatment. Dr. Berdy is continuing to contribute information on new compounds each year to this file which is maintained in computerized form in Frederick and in card index form in Budapest. There are now over 5000 entries in the file. There are an increasing number of inquiries from non-Government Agencies for infomration contained in the file and discussions were conducted with Dr Berdy concerning the possibility of transferring the data of the Berdy Classification into the International Cancer Research Data Bank.. This appears to be quite feasible.

8. (With J. Ziegler and O. Yoder) attended the Institute of Cancer Research conference at the University of Kent in Canterbury, England on April 4-6, 1977. Attendance at the conference was in response to invitation from Professors Tom Symington, Director of the Institute of Cancer Research and Dean L. F. Lamerton. The program was in the form of a Scientific Retreat involving approximately 200 staff members from the various components of the Institute of Cancer Research: Royal Cancer Hospital including the Chester Beatty Institute, The Royal Marsden Hospital and the Pharmacologic and Clinical Programs in Sutton. Attendance at this program provided a unique opportunity for a complete review of the Institute of Cancer Research's ongoing program at first hand and to discuss various facets of the program with the responsible investigators. We currently have a drug collection and evaluation contract with the Institute of Cancer Research for new antineoplastic agents developed in the United Kingdom and serious consideration will be given to the possibility of expanding this contract to encompass total program collaboration ranging from synthesis and initial evaluation of the effectiveness of antitumor agents

Postgraduate Medical Education, Warsaw; Province of Katowice;and the United Pharmaceutical Industry in "Polfa", Warsaw. The course involved formal presentation and round-table discussion. The latter included:

A. Objective Evaluation of Effectiveness of Chemotherapy
B. The Place of Chemotherapy in Cancer Management
C. Chemotherapy: How aggressive should it be?

The round-table discussions as well as the more formal presentations will be translated into Polish and published in a Polish Chemotherapy Journal. During the round-table discussions as well as in discussions pertaining to individual papers there was strong emphasis on the importance of collaboration between preclinical and clinical investigators. The EORTC has established a definitive relationship with the COMECON (Chemotherapy Program in the Socialist Countries) which in turn will be very useful in supplementing our own program in the U.S.

5. On January 21-24, participated in an "International Conference of Experts on Cancer Research" which was organized and sponsored by the German Foundation for Cancer Research. Drs. G. O'Conor and A. Goldin were the two representatives at this meeting from the National Cancer Institute. The meeting was conducted under the initiative of Dr. Mildred Scheel, the wife of the President of the Federal Republic of Germany. The objectives of the conference involved an attempt to develop a general concept for cancer investigation in Germany in relation to present and future aspects of research. There is a great potential for collaboration with the Cancer Therapy investigators in Germany and the conference provided a good opportunity for Drs. O'Conor and Goldin to discuss with various German participants and members of other countries matters pertaining to International Collaborative NCI Programs. In addition discussions were held by A. Goldin with Professor Schmidt and Dr. Osieka concerning the development of a preclinical - clinical program involving therapy of human tumors xenografted into athymic mice. Professor Schmidt's institute in Essen is specifically interested in such collaboration and there is an excellent opportunity for development of program in this area.

6. Attended a meeting of the EORTC Council in Brussels on March 19, 1977 at the invitation of the Council. This provided an opportunity to keep abreast with the numerous activities and developments in cancer treatment investigation in Europe as related to the International activities of the Division of Cancer Treatment. The EORTC now has thirty-four cooperative groups, clubs, working parties etc. and these involve several hundred clinicians in hospitals in ten countries. There are forty-two active clinical protocols at the present time. The EORTC now has thirteen hundred subscribers to its Newsletter.

On March 21-23, 1977 (with O. Yoder) attended an International Symposium on Ifosphamide in Dusseldorf Germany. This international symposium was organized in view of the entry of ifosphamide (Holoxan) into the German market. The international symposium covered both preclinical and clinical aspects of the investigations with ifosphamide. The drug ifosphamide is different from cyclophosphamide in a number of its pharmacologic and therapeutic actions and is clearly active in the clinic.

to preclinical and clinical pharmacology, toxicology and Phase I and II clinical trials.

The following talks describing various aspects of our program were given at International conferences during the year by A. Goldin.

1. "The Preclinical Program of the National Cancer Institute" presented at a seminar on preclinical and clinical programs of the National Cancer Institute in Perugia.

2. "Potentiality of Tumor Xenografts in Nude Mice" presented at the International Union Against Cancer Symposium on "Human Tumor Sampling and Biochemical Pharmacological Studies of Target Determinants of Drug Action" in Moscow.

3. "Resistance to Antitumor Agents" presented at the International Symposium on "Recent Advances in Cancer Treatment" in Brussels.

4. "Search for New Radiation Potentiators" presented at the Sir Stanford Cade Memorial Symposium on the "Interaction of Radiation and Antitumor Drugs." at the Royal Institution in London.

5. "The Value of Experimental Models for Prediction of Clinical Effectiveness" presented at the "Cancer Chemotherapy Course" in Wisla, Poland.

6. "Preclinical Therapy" presented at the International Conference of Experts on Cancer Research in Bonn.

7. "Preclinical Investigations with Ifosphamide in Relation to Cyclophosphamide" presented at an International Symposium on Ifosphamide (Holoxan) in Dusseldorf, Germany.

8. "Experimental Basis of Combination Chemotherapy" presented at the VII International Symposium on the "Biological Characterization of Human Tumors: in Budapest, Hungary.

9. "The Preclinical Development Program of the National Cancer Institute in Bethesda" presented at the Institue of Cancer Research Conference at the University of Kent in Canterbury, England.

10. "Criteria for Selection of New Analogs of Antitumor Antibiotics" presented at the Joint US-Japan Meeting in San Francisco, California.

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PUBLICATIONS SECTION

Office of the Director

The Publications Section directs the preparation of Cancer Treatment Reports (CTR) (the principal scientific journal developed under the DCT Program) and provides editorial assistance to the DCT staff on a time-available basis.

CTR (formerly Cancer Chemotherapy Reports) is now in its 18th year of continuous publication. From 1959 to 1968 CTR was issued 6-10 times a year depending on the acceptance of manuscripts submitted. Several types of manuscripts were published including program information, study protocols, experimental studies, and clinical reports. Most, but not all, manuscripts were reviewed by outside referees as well as the editorial board. In 1968 the journal expanded to three parts: Part 1 (reviewed manuscripts on original research, both experimental and clinical), Part 2 (comprehensive, lengthy chemotherapy studies involving a great deal of tabular material; some of the material was reviewed by referees), and Part 3 (program information including study protocols, clinical brochures, toxicology reports, and review articles; this material was normally not reviewed). In January 1976, CTR dropped the three-part system and began publishing monthly. In addition, the journal will now review manuscripts related to all areas of cancer treatment: radiation, surgery, immunotherapy, chemotherapy, and combined modalities. All original research material is reviewed. Every other issue now contains the proceedings of a symposium or seminar.

Submissions in 1976 and 1977

During 1976 the journal received 327 research manuscripts and 151 symposia manuscripts. In the first 5 months of 1977, 145 research manuscripts and 120 symposia manuscripts were submitted.

Comparative data on the CTR series of publications are shown in table 1. The manuscripts accepted are reported monthly to the Editorial Office of the Journal of the National Cancer Institute, which in turn distributes this information in their monthly reports.

Sections of the Journal

Brief Reports.--In 1973, a section entitled Brief Reports and Preliminary Communications was added to the journal. Authors were invited to submit short clinical notes or the abbreviated results of preliminary work. These reports have been processed through the editorial channels more quickly than full-length manuscripts, thus reducing costs for production time and allowing more such manuscripts to reach the readers. The response to this new section has continued to be excellent during the past year. Each issue has contained 5-10 Brief Reports and an increasing number are being submitted.

Table 1

Year	Part	Mss rec'd	Mss accepted	Issues published	Pages published	Reviewers	Total costs ($)*	Cost per page ($)
1972	1	122	76	6	814	197	37,715	52
	2	4	5	1	353		21,064	
	3	47	47	2	181		10,863	
		173	128	9	1348		69,642	
1973	1	148	112	4	524	167	23,713	54
	2	19	19					
	3	77	76	3	576		26,184	
		244	207	7	1100		59,897	
1974	1	244	138	6	978	212	42,706	40
	2	21	21	4	758		25,069	
	3	21	21	1	87		4,550	
		286	180	11	1823		72,325	
1975	1	215	114	6	1206	295	62,315	51
	2	19	19	1	263		11,374	
	3	131	138	2	419		21,809	
		365	261	9	1888		95,498	
1976		478	302	12	2021	402	100,210	49
1977**		265	279	9 (scheduled)	ca 1600	--	Not available	--

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*Includes the cost of printing, typesetting, and distribution only.

**From Jan 1 to June 15, 1977.

Commentaries.--In 1974, a section containing invited editorials and commentaries was initiated; in the past 2 years, many such manuscripts have been received and published. An authoritative scientist or physician is invited by a member of the Editorial Board to write briefly on a subject of current interest in cancer chemotherapy. We hope this section will encourage more interest in current topics, particularly through the submission of Letters to the Editor. In the past year we have accepted commentaries on the following topics: "Advances in Small Cell Bronchogenic Carcinoma" and "Whatever Happened to NSC ------? An Analysis of Clinical Results of Discontinued Anticancer Agents."

Letters to the Editor.--During the past year CTR has observed a significant increase in the number of Letters submitted. These Letters usually, but not always, refer to previously published articles. Between May 1976 and June 1977 we have accepted or scheduled for publication 26 Letters.

Proceedings of Symposia

During the past several years, CTR has been approached frequently to publish the proceedings of scientific meetings. In 1976, the proceedings of 7 symposia were published, and in 1977 we have published (or will publish) the proceedings of the following meetings:

1. National Prostatic Cancer Project Workshop (Roswell Park Memorial Institute)
2. Antimetabolites and the Central Nervous System (Sloan-Kettering Cancer Center)
3. The Non-Hodgkin's Lymphomas (National Cancer Institute and the Cancer Clinical Investigation Review Committee)
4. Osteogenic Sarcoma Study Group Meeting (National Cancer Institute)

We have so far scheduled to publish the proceedings of the following meetings during 1978:

1. The Use of Athymic (Nude) Mice in Cancer Research
2. Charles P. Perlia Memorial Symposium on Breast Cancer (unconfirmed)

Cumulative Index

In 1975 the editorial staff began compiling a 10-year subject and author cumulative index for CCR Part 1 (1966-1975) and an index for all material published between 1968 and 1975 in Parts 2 and 3. Because of the extensive work required, the Publications Section contracted the completion of this work to a biologic abstractor with extensive experience in cancer research. The index has now been completed and is currently being processed. It will be published in late 1977.

Official Charter for CTR

In April 1975, the first charter meeting of the CTR Editorial Board was held. This meeting was intended to resolve several issues concerning the organization of CTR, the name change to reflect the new direction of the DCT, and the additional duties of the Publications Section. In addition, the Board has written a substantive charter outlining such technicalities as the term of office for editors and the editor-in-chief (3 and 4 years respectively), the official policy regarding review and rejection, and the specific areas of research to be covered.

Editorial Board

In 1975 the Editorial Board expanded to include nine associate editors in addition to the editor-in-chief. Each year three editors will rotate off the board and three new members will be added. Provisions have been made to allow an additional year of editorship at the discretion of the editor-in-chief and the Director, DCT. In 1976, an Editorial Advisory Board with 15 members was established. The members will serve in a peripheral way to supplement the areas of expertise represented by the associate editors. Their advice regarding policy decisions, acceptance of symposia, and new areas to be covered will be solicited.

Coverage of CTR in Current Contents and Related Publications

Since 1967 CTR has been listed in Current Contents, Life Sciences. In 1973, CTR was asked to be included in a new publication of current titles in collaboration with Science, Engineering, Medical and Business Data Ltd., Oxford, England, and in 1974, we were asked to establish a similar policy with the Japan Medical Service, which publishes a supplement to its Index of Japanese Medical Periodicals listing foreign publications. It is expected that this additional coverage of the material presented in the journal will increase the demand for subscriptions, particularly in other countries.

In 1964, CTR began sending proof copies of each issue to the Chemical Abstracts Service for abstracting and indexing of the chemical information. During 1973 CTR was asked to establish a similar policy with the Biosciences Information Service (Biosis) in Philadelphia, and with Infordata International in Chicago, which published the Index to the Periodicals of the US Government.

Office of Management and Budget Approval

In late 1976 we submitted to the OMB our request for continuation of funds. This document outlined the purpose and scope of CTR, the publications costs incurred by the staff, a breakdown of the distribution of each issue (including the categories of subscribers), and the justification for continuing the publication. In early 1977 we received OMB approval which allows us to continue production through January 1980, at which time we will be required to request further funding.

Distribution of CTR

The Publications Section is responsible for maintaining the free mailing list for CTR. CTR is available without cost to qualified medical groups, physicians, and libraries, and is for sale to others by the Superintendent of Documents, Government Printing Office. The approximate distribution, which varies with each issue, is as follows:

3225	Foreign and domestic free mailing
1800	Superintendent of Documents (for paid subscriptions)
367	Superintendent of Documents (for Depository Libraries)
5392	

In the past several years, the number of paid subscriptions has increased tremendously. The number will undoubtedly continue to rise because of the lower limits set by the OMB each time the journal receives OMB clearance. The demand for specific back issues remains high; most requests are for the proceedings of meetings.

Problems with the service rendered by the NIH Distribution Section in maintaining the mailing list have brought about a change in the distribution procedures. Beginning in 1976 maintenance of the mailing list was transferred to the Publications Section, and is now in a computerized format. This conversion allowed more rapid service in 2 areas: (a) adding, deleting, and changing names on the list, and (b) obtaining mailing labels to be used by the contract printer in mailing the issues to the subscribers. In 1977 we screened the mailing list to eliminate addresses of subscribers who have moved, retired, or died. This clearing of the mailing list allowed us to add names of many individuals that were on a waiting list.

LITERATURE RESEARCH SECTION

Office of the Director

The Literature Research Section serves the Division of Cancer Treatment by providing information from published literature on all aspects of the therapy of cancer. Data from the fields of chemotherapy, radiotherapy, surgery, immunotherapy and the related chemical and biomedical disciplines are used by the staff in Decision Network review, meeting FDA requirements for IND filing, preparing clinical brochures, and as background for evaluation of toxicological and clinical studies.

Literature Services. The Section received and filled over 375 requests for information during the reporting period. Responses were provided as comprehensive or selected bibliographies, computer print-outs, abstracts and copies of articles. Almost half of the requests entailed manual literature searches supplemented by the various automated bibliographic retrieval systems such as Medline, Toxline and Cancerline. Some of the extensive clinical searches performed were for single and combined drug therapy of lymphomas, treatment of breast and endometrial cancer, neurological and cardiotoxicity of antineoplastic agents, and the effects on progeny when chemotherapy was administered during pregnancy. Bibliographies were prepared, or updated, for such agents as bleomycin, BCG, streptozotocin, isoxazoleacetic acid, bruceantin, deoxycoformycin and nitroimidazole radiosensitizers. Searches restricted in scope were done for aspects of treatment of various neoplasms and for specific effects of drugs under consideration for, or already in, development.

The Section co-ordinates NCI access to the on-line data bases of the Medlars automated bibliographic search system. In this role the Section serves all areas of the Institute, processing searches and providing assistance and instruction in the use of the system. It is also the center for access to the Toxline and Cancerline data bases for personnel in the Blair Building. These computer services aid in the searching and facilitate the answering of requests. Responses to more than 125 requests were in the form of computer print-outs only. Monthly SDI (Selective Dissemination of Information) bibliographies are produced for members of the staff on specific subjects of continuing interest.

The staff continues to be responsible for the preparation and distribution of the agent folders for Decision Network meetings. Each folder contains the structure and systematic name of the compound, a bibliography from the published literature, summaries of screening, toxicological or other preclinical or clinical data, and questionnaires. Materials were assembled for a total of 54 drugs.

A further responsibility of the Section is the maintenance of the Cancer Therapy Library, a collection of books and journals for the use of NCI staff. Copies of over 60 journals are regularly received including many of the cancer journals, abstracting and indexing secondary sources, and chemical, biomedical or information science journals of special interest to the Section and other personnel in the building. Approximately $11,000 was spent on books and subscriptions.

Contract Operations. Contracts for the preparation of Cancer Chemotherapy Abstracts and its successor Cancer Therapy Abstracts have been monitored by members of the Literature Research Section since 1965. In addition to the printed journal and hard copy of all cited articles, computer tapes of the abstracts and indexes are received from the contractor. Under the sponsorship of the International Cancer Research Data Bank, these are input to the Cancerline file which can be searched on-line through the Medlars network.

SUMMARY REPORT OF THE DEVELOPMENTAL THERAPEUTICS PROGRAM

DIVISION OF CANCER TREATMENT

NATIONAL CANCER INSTITUTE

The Developmental Therapeutics Program (DTP) was established during the past year through the consolidation of the Drug Research and Development Program and the Experimental Therapeutics Program. This amalgamation of two preclinical programs with centralization of program direction into one office was prompted by the urgent need to realize more rapidly the program goals of the Division of Cancer Treatment (DCT). As one of four major subdivisions of the DCT, the DTP is composed of a large intramural laboratory research operation and a very much larger extramural contract program directed towards the preclinical development of therapeutic modalities, in particular those related to chemotherapy of malignant disease. The primary task of the DTP is to support and conduct the search for potential cancer therapeutic agents and subject them to programmed preclinical evaluation according to a scheme called the Linear Array. It is responsible for all preclinical phases of drug development.

The total intramural and extramural Program is distributed among nine discrete, but closely integrated, in-house laboratories and branches and the Office of the Associate Director: (1) Laboratory of Chemical Pharmacology; (2) Laboratory of Medicinal Chemistry and Biology; (3) Laboratory of Molecular Pharmacology; (4) Laboratory of Toxicology; (5) Laboratory of Tumor Cell Biology; (6) Drug Evaluation Branch; (7) Drug Synthesis and Chemistry Branch; (8) Natural Products Branch; and (9) Pharmaceutical Resources Branch. The research activities are concerned with the design, synthesis, evaluation and mechanism of action of antitumor agents, the pharmacological and toxicologic properties of drugs at the molecular, cellular, and whole body levels, the development and use of biologic markers to assess tumor growth, and the molecular biological aspects of neoplastic transformations.

The extramural contract program is principally concerned with the following activities: a) the acquisition of new drugs, synthetic materials, plant products, fermentation products and other natural products for evaluation as potential agents; b) the evaluation of the drug in a panel of tumor screens; c) the procurement and distribution of animals for primary screening and evaluation of new materials; d) the development and production of clinical dose forms for clinical trial; e) pharmacologic and toxicologic evaluations at the pre-clinical and clinical levels; f) investigations of new approaches to combined modality therapies, involving surgery, radiotherapy, and immunotherapy in addition to chemotherapy; and g) the development and operation of data processing systems for both biological and chemical information.

Programmatic Changes and Accomplishments

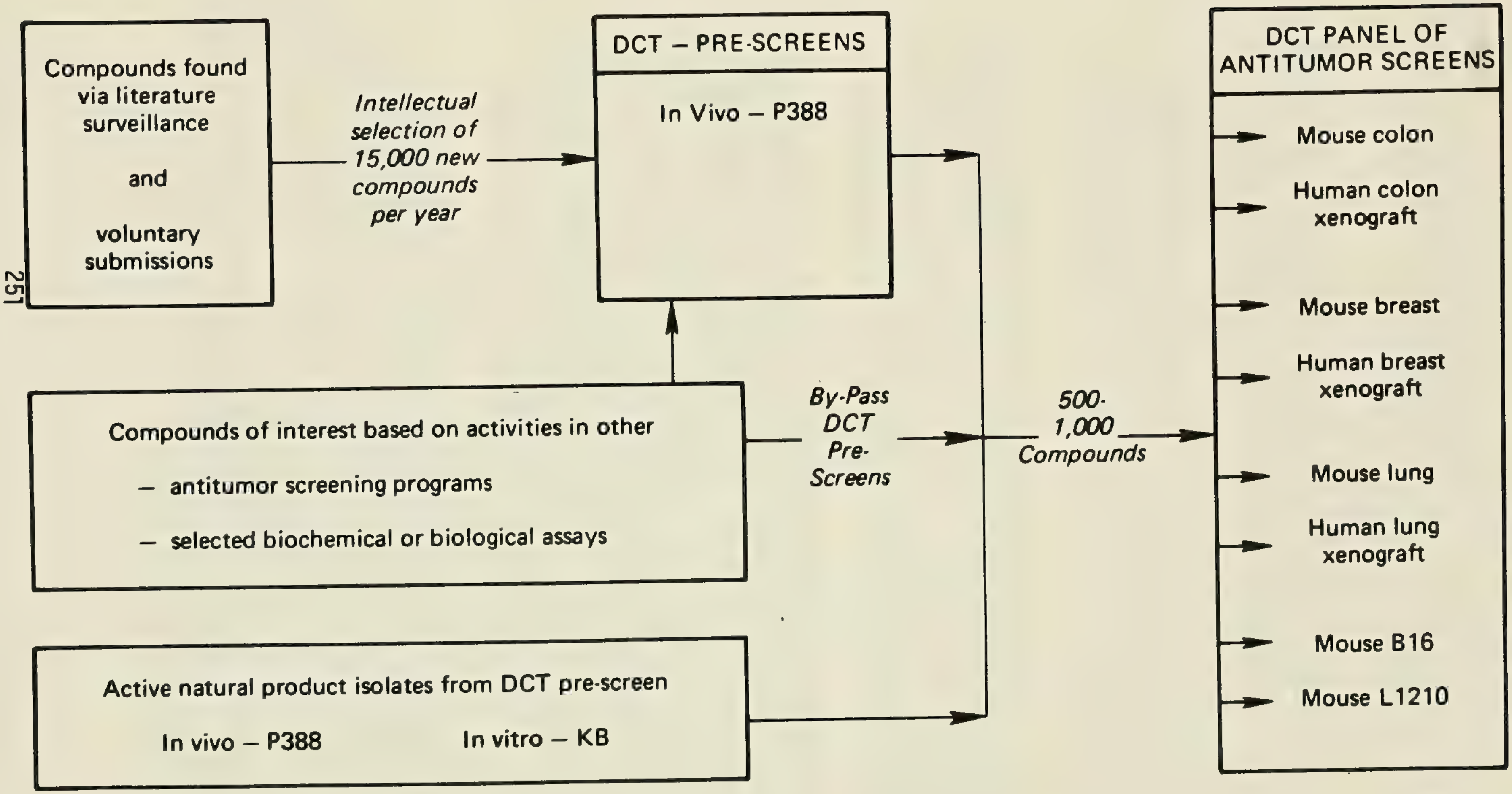
The following highlights the changes and/or accomplishments of the past year which resulted from decisions made at the Program level and which impact across the whole range of the drug development process:

1. Prioritization of Agents in the Decision Network Apparatus.—At the request of the Director, DCT, several special decision network (DN) meetings were held to review and reassess the approximately 60 agents currently in various preclinical or clinical testing stages. As called for by the Program Logic described described in the Linear Array, the DN meeting is customarily the mechanism used to consider whether certain criteria have been met for moving candidate antitumor agents from one stage of development to another. The special DN meetings were held with the express purpose of expediting as much as possible the flow of promising high priority compounds through the various stages of drug development into clinical trial. They were also to assign a priority status to all agents. Those judged of little promise were slated for removal in order to conserve limited program resources. As a result of the first of these meetings, the following compounds were dropped from further consideration: Chromomycin A_3 (NSC-58514), Fluorodopan (NSC-73754), d-Tetrandine (NSC-77037), Cytembena (NSC-104801), "Discreet" Compound (NSC-124921D), Coralyne Sulfoacetate (NSC-154890), Asaley (NSC-167780), Quinolinium (NSC-176319), Sterigmatocystine (NSC-204985), Ledacrine (NSC-247561), Pure Cesalin (NSC-264879), Butocin (NSC-172755), Thalicarpine (NSC-68075), and S-Trityl-L-Cysteine (NSC-83265).

At a subsequent meeting, the agents remaining in the system were assigned to a "high", "moderate", or "low" priority status with the prospect that in the very near future of a number of compounds in the latter category will also be removed from active development in the Drug Development Program.

2. Status of DCT Screening Systems.—The past year saw a greater emphasis on the testing of new synthetics and natural product isolates against a spectrum of animal tumor models. A selection was also made of specific human tumors (colon, lung, breast) to be grown as xenografts in athymic (nude) mice to complete the secondary evaluation panel (Figure 1) of antitumor screens for the evaluation of the projected annual input of 500-1,000 compounds. The switch from the leukemia L1210 primary screen to the *in vivo* leukemia P388 pre-screen was completed and the erythroleukemia model as a potential ancillary *in vitro* pre-screen was dropped because it was judged inappropriate for large-scale screening. Currently, the leukemia L1210, B_{16} melanoma, Lewis lung carcinoma, and colon tumor 26 screens in the secondary panel can be fully occupied by the projected 500-1,000 compound submissions per year while the mouse CD8F1 mammary carcinoma is expected to have the capacity to screen 250 compounds during the next year. It is also expected that within the next year, a submission capacity of at least 250 compounds will be reached by each human tumor xenograft screen. A more detailed discussion of this topic is contained in the Summary Report of the Drug Evaluation Branch. To implement the changes in the DCT screening systems, the input of new synthetic compounds into the P388 has been reduced to 20,233 in 1976 as compared to 21,295 in 1975, towards the goal of 15,000 annual submissions. Active efforts are also underway to define, in the next several months, pre-selection criteria for the P388 pre-screen and

FIGURE 1. FLOW OF DRUGS THROUGH DCT SCREENS


Compounds found via literature surveillance
and
voluntary submissions
Intellectual selection of 15,000 new compounds per year
DCT – PRE-SCREENS
In Vivo – P388
Compounds of interest based on activities in other
– antitumor screening programs
– selected biochemical or biological assays
By-Pass DCT Pre-Screens
Active natural product isolates from DCT pre-screen
In vivo – P388
In vitro – KB
500-1,000 Compounds
DCT PANEL OF ANTITUMOR SCREENS
Mouse colon
Human colon xenograft
Mouse breast
Human breast xenograft
Mouse lung
Human lung xenograft
Mouse B16
Mouse L1210

the biological and biochemical criteria to by-pass the P388 pre-screen. A more detailed discussion of the input of the number of synthetic, plant, animal, and fermentation products into the Program during the past year, as well as efforts to establish pre-selection criteria, is summarized in the Annual Report of the Drug Synthesis and Chemistry Branch.

3. Program Output of New Agents.—During this reporting period, 15 compounds (Table 1) passed DN 2A, having demonstrated sufficient reproducible antitumor activity and/or other unique chemical or biologic properties in experimental systems to warrant dose formulation development and preclinical toxicology studies. Pentamethylmelamine (NSC-119742), dihydro-5-azacytidine (NSC-26488), aziridinylbenzoquinone (NSC-182986), and two adenosine deaminase inhibitors, 2-deoxycoformycin (NSC-218321) and EHNA (NSC-263164) which potentiate the activity of arabinosyl adenine (NSC-404241) were among those agents which have been assigned a high priority status in order to guarantee the special attention needed for rapid further development by the Program.

In the pre-clinical toxicology area, full protocol studies were completed on six new agents plus adriamycin and abbreviated/special studies completed on 16 additional compounds as shown in Table 2. Another nine agents are currently undergoing full protocol or abbreviated toxicologic studies. These studies constitute a vital part of the pre-clinical data input on drugs which is required before DCT can file for an investigational new drug application (INDA) approval from the FDA to conduct initial clinical Phase I trials.

4. New Research and Development Projects with Contract Support.—Within the past reporting period, competitive bids were received on request for proposals (RFP) for research and development projects in two specific areas, the liposomal encapsulation of antitumor drugs (RFP 67118W) and the synthesis of radiosensitizing agents (RFP 77139). Both projects had earlier received conceptual approval by the DCT Board of Scientific Counselors as new areas for exploitation by the Division through contract support. The former project reflects the Division's interest in exploring novel *in vivo* drug delivery systems with the aim of ameliorating toxicity to vital target organs and tissues and to enhance the therapeutic efficacy of the parent drug. The latter project represents the initial phase in developing agents which can enhance the therapeutic effects of radiation in the treatment of malignant disease. Award of several contracts to conduct these projects is imminent.

5. Organization of Drug Analog Committees.—As part of the Division's new approach to managing the analog synthesis program, analog coordinating groups were organized to make recommendations to the Division Director and at DN meetings and to serve as advocates for their choices of drugs to be developed with the Division's resources. DTP personnel played an important role in organizing the groups and conducting initial meetings during the past reporting period. Five of the seven analog coordinating groups are chaired by senior staff members of the DTP, i.e. the Coordinating Groups for analogs of platinum compounds, actinomycins, alkylating agents, nucleosides, and antifols.
In addition, numerous members of the DTP scientific staff serve as members on all of the Coordinating Groups.

TABLE 1

Compounds Passing Decision Network 2A

NSC No.	Compound
118742	Melamine, N2,N2,N4,N4,N6-pentamethyl-, monohydrochloride
141401	Poly(p-dioxane-2,5-diyloxyethyleneiminohexamethyleneiminoethyleneoxy)
153353	L-Alanosine, monosodium salt
164011	Rubidazone
177023	Levamisole
182986	1,4-Cyclohexadiene-1,4-dicarbamic acid, 2,5-bis (1-aziridinyl)-3, 6-dioxo-diethyl ester
191959	Michicarcin
218321	Imidzao[4,5-d][1,3]diazepin-8-ol, 3-(2-deoxy-β-D-
219877	Rachelmycin
240899	5-Benzofurancarboximidamide, 2,2'-(1,2-ethenediyl) bis-, dihydrochloride
259968	Bouvardin
263164	9H-Purine-9-ethanol, 6-amino-β-hexyl-α-methyl-, monohydrochloride STE (R*,S*)-
264880	1,3,5-Triazin-2(1H)-one 4-amino-3,6-dihydro-1-β-D-ribofuranosyl-monohydrochloride
282175	4-Hydroxyandrost-4-ene-3,17-dione
283162	Methanol, [1,3,5-triazine-2,4,6-triyltris(methylimino)]tris

Table 2

Protocol Toxicity (Task I) Studies Completed 1976-7

Compound	NSC Number
Coralyne Sulfoacetate	154890
Chlorozotocin	178248
Quinolinium Dibromide	176319
Methanesulfon-m-anisidide	249992
Bruceantin	165563
Pyrazolo-imidazole	51143
Adriamycin	123127

Abbreviated/Special Toxicity (Task II) Studies Completed 1976-7

Compound		NSC Number
DL-Alanosine	*	143647
L-Alanosine		153353
5-Fluorouracil	*	19893
Fluorodopan		73754
Ftorafur		148958
Diglycoaldehyde	*	132313
Bleomycin		125066
BCNU		409962
Hexamethylmelamine		13875
MeCCNU		95441
Chromomycin A_3		58514
Discreet Compound		124921
Discreet Compound		124920
Spirogermanium		192965
Nitidine		146397
Tetrahydrouridine		112907

*Comparative study

6. Biologic Markers Program.—The DTP was successful during the latter part of this reporting period in recruiting a distinguished physician-scientist and former staff member of the NCI-NIH community to head the Biologic Markers Program for the Division. Despite the absence of a Coordinator for the Program for more than a year, considerable progress was made by our contractors in their efforts to develop and test markers for human malignant disease.
A major segment of this Program resides at the Biological Markers Laboratory at the Frederick Cancer Research Center (FCRC) which was established under contract in late 1975. This facility consists of: 1) a Specimen Repository for receiving and storing body fluids and other biological samples under appropriate conditions from selected clinical studies and for normal control specimens as well as samples from patients with diseases other than cancer; 2) an Analytical Section to carry out routine marker analyses for specific tumors and collaborate with the markers basic research program (at Frederick, NCI, and NCI-supported facilities) in the development of marker analysis methodology; 3) a Biological Markers Research Section to conduct research for both the development of new and the improvement of existing specific tumor markers; and 4) a relatively small Drug Metabolism Section to carry out collaborative pharmacologic disposition studies on agents of interest to the NCI where advantage is taken of the Analytical Section for identification of drugs and metabolites in biological specimens. The recruitment of scientific personnel for all phases of the Program was completed during the past year.

Collaborative clinical studies are underway or about to be initiated between the FCRC unit and the NCI (Breast, Ovarian, Testicular, Gastrointestinal and Lung Cancer Protocols), Johns Hopkins (Ovarian and Small Cell Carcinoma Protocols), and Mayo Clinic (Breast and Gastrointestinal Cancer Protocols). Potential bio-markers under study at all contractor facilities include carcinoembryonic antigen, human chorionic gonadotropin β-subunit, degradation products of tRNA (N^2,N^2-demethylguanosine, β-aminoisobutyric acid, etc.), polyamines, protein bound serum carbohydrates (sialic acid, fucose, etc.), aryl sulfatase, histaminase, calcitonin, sialyl-, fucosyl-, and galactosayl-transferases, a glycoprotein hormone, DNA-binding proteins, terminal deoxynucleotidyl transferase, and others.

Individual Intramural Laboratory Research Contributions

Some of the intramural laboratory research accomplishments are highlighted below. Further details may be obtained from each of the annual reports of the Laboratory in which the investigation was undertaken.

1. An exciting new agent, N-(phosphonacetyl)-L-aspartic acid (PALA), a rationally synthesized inhibitor of aspartate transcarbamylase, was shown in the Laboratory of Chemical Pharmacology (LCP) to be active in a broad spectrum of experimental solid tumors, but inactive in several murine leukemias. The compound has been given a high priority status for rapid development by the Program.

2. Extending recent developments by the pharmaceutical industry, the Laboratory of Medicinal Chemistry and Biology (LMC&B) and the LCP in a collaborative effort have been investigating the biological, biochemical, and pharmacological properties of two agents which act as potent inhibitors of the mammalian enzyme adenosine deaminase. Evidence has been accumulated to show that the antitumor activity of several adenosine analogs is potentiated by the coadministration of these inhibitors, 2'-deoxycoformycin (NSC-218321) or erythro-2-(2-hydroxy-3-nonyl)-adenosine (EHNA, NSC-263164), through inactivation of the enzymatic deamination process. The Division is planning to pursue promptly these observations in an early clinical trial involving 2'-deoxycoformycin and adenine arabinoside 5'monophosphate (NSC-259272).

3. Studies by the Laboratory of Molecular Pharmacology (LMP) to define the types of DNA damage to cells caused by alkylating agents have progressed to the point where four types of DNA damage can be identified: single-strand breaks, alkali-labile sites, DNA-protein links, and DNA interstrand crosslinks. Based on differences between the kinetics of the formation of DNA-protein links and DNA interstrand crosslinks by nitrogen mustard and melphalan, an important new investigational approach is being promulgated by the LMP termed "intracellular pharmacokinetics." Such an approach would measure the rates of appearance and disappearance of specific macromolecular lesions at drug reaction sites which then could be related to cell lethality.

4. The Laboratory of Tumor Cell Biology (LTCB) reported the first successful attempt to establish in culture a continuously replicating leukocyte line from the blood of a patient with acute promyelocytic leukemia. These cells can grow in the absence of conditioned media while showing continued evidence of myeloid differentiation. This provides a culture system with the potential for the rapid assay of those chemotherapeutic agents which can selectively exert their cytotoxic effects against this leukemia.

5. To facilitate pharmacologic and molecular biology studies of spirohydantoin mustard (SHM, NSC-172112), a compound first synthesized as an organ specific antitumor agent in the LMC&B, a quantitative gas chromatographic-mass spectrometric method was developed in the LCP to measure nanogram levels in the CSF and blood after parenteral injection in animals. SHM originally showed curative activity in the mouse intracranially implanted ependymoblastoma. This activity correlated with its ability to cross the blood-brain barrier unchanged when it achieved significant but low concentrations in the CSF.

6. Another important development by the LTCB was the isolation of antibodies from short-term cultures of leukocytes from CML patients which possess high neutralizing activity against feline and murine virus reverse transcriptase (RT). In addition, antibodies isolated from AML patients possessed high activity against RT from the HL 23 virus previously isolated from leukocytes of a patient with AML.

7. The LMC&B is continuing studies on the mechanism of action of the ansa macrolide maytansine, a new agent recently developed by our Program and which is currently in early Phase II clinical trials. It was demonstrated that maytansine, similar to the action of vinca alkaloids, impairs the formation of microtubules in mammalian cells and also competes with the vincas for the same binding sites on tubulin in mammalian brain.

8. As part of their interest to develop markers of neoplastic cells for quantitating residual tumor cells after therapy and in remission, the LTCB demonstrated the presence of terminal deoxynucleotidyl transferase in CML patients in the acute blast phase of the disease, its loss during treatment, and reappearance before blast crisis.

9. The LMC&B reported that the heretofore unsuccessful attempts to synthesize the reduction product of the antitumor agent 5-azacytidine have led to the preparation of a more chemically stable 5,6-dihydro-5-azacytidine with antitumor activity comparable to the parent compound in several *in vivo* and *in vitro* experimental systems.

10. The Laboratory of Toxicology (LT) reported that L-alanosine (NSC-153353), a new antitumor agent destined for clinical trial, but currently in preclinical toxicologic evaluation, appears to be primarily metabolized to an α-hydroxy analog which does not possess the cytotoxic potency of the parent compound. The results indicate that L-alanosine itself is the "active" moiety and is thought to act by inhibiting the activity of adenylosuccinate synthetase leading to depletion of AMP, ATP and dATP pools.

11. The LMC&B has also recently reported that the cytotoxicity of the important antitumor agent melphalan (phenylalanine mustard, L-PAM) to L1210 leukemia cells in culture is reduced in growth medium containing the L-isomers of leucine and glutamine. The decreased cytotoxicity is a result of decreased transport into the amino acid protected cells with L-leucine sharing a common transport system but with higher affinity for common intracellular binding sites. These observations have been extended to *in vivo* studies in rodents where the simultaneous administration of leucine interferes with the activity of therapeutic doses of L-PAM and studies have been initiated to test the clinical implication of these investigations in collaboration with the Medicine Branch in the Clinical Oncology Program.

ANNUAL REPORT OF THE LABORATORY OF CHEMICAL PHARMACOLOGY

DEVELOPMENTAL THERAPEUTICS PROGRAM

NATIONAL CANCER INSTITUTE

July 1, 1976 - September 30, 1977

Studies in the Laboratory of Chemical Pharmacology are concerned with the rational design and evaluation of antitumor agents and with elucidation of the pharmacological properties of established as well as new antitumor agents. These studies provide information on the antitumor activity in model systems, the pharmacological disposition and ability to penetrate the CNS, and dose-response relationships of antitumor drugs and other model foreign compounds. Parallel studies involve defining the mechanism of action and structure-activity relationships of these drugs, as well as evaluating their toxicity and designing techniques for reversing such toxicity. In many instances, these studies require the development of new methodologies to facilitate pharmacologic studies of antineoplastic agents whose disposition is not fully understood. In disposition studies, particular emphasis is given to establishing those rate constants which govern the passage of drug across membranes from one body compartment to another, to assessing drug biotransformation or binding to cellular macromolecules or other constituents, and to determining the rate of elimination of the drug and its metabolites. These studies are important, since one of the major factors contributing to an inadequate drug response in the host is the failure of the drug to reach the target site in sufficient concentration for a sufficient period of time to eradicate the tumor cells. The knowledge gained from such studies, when considered together with biochemical, toxicologic, and cytokinetic data, should enable the clinician to utilize drug dosages and schedules for the most effective treatment of human malignancies.

During the past year several research projects were continued and a number of new studies were initiated. Some of the experimental findings are summarized below.

Several new antitumor agents and analogs of some clinically active antitumor agents were evaluated _in vitro_ and in several rodent tumor systems. The potent adenosine deaminase inhibitor, 2'-deoxycoformycin, inhibited the enzymic inactivation and potentiated the cytotoxic activity of a variety of adenosine analogs in the P388 murine leukemia cell culture system. Six of the seven adenosine analogs examined showed enhanced cytotoxic activity when combined with 2'-deoxycoformycin; the exception was tubercidin (7-deaza-adenosine), which was not a substrate for the deaminase. In studies in mice, 2'-deoxycoformycin and another adenosine deaminase inhibitor, erythro-9-(2-hydroxy-3-nonyl)-adenine (EHNA), potentiated the antineoplastic activity of 9-β-D-xylofuranosyladenine against P388 leukemia. In addition, 2'-deoxycoformycin was shown to have immunosuppressive activity in mice using an allograft tumor test system.

During the past three years, a comparative evaluation of the antitumor activity of 35 actinomycin analogs has been carried out using a variety of experimental tumors. None of these analogues had clear therapeutic superiority over the parent antibiotic, actinomycin D, although marginally better antitumor activity of actinomycin $\mathrm{Pip}_{1\beta}$ was demonstrated and repeatedly confirmed in B16 melanoma. Considerable information on the structure-activity relationships among actinomycins was obtained which will be useful in further efforts to develop a superior actinomycin analog.

The high degree of antitumor activity of anguidine in P388 leukemia and particularly in colon carcinoma 38 has aroused a good deal of clinical interest in this mycotoxin. A closely related trichothecene, T2 toxin, and several macrocyclic analogues of this group of compounds were compared in experimental tumor systems. T2 toxin was reproducibly superior to anguidine in P388 leukemia as were three macrocyclic derivatives from *Baccharus megapotamica*, baccharin, B_1 and B_2. In addition, the latter two compounds were quite active against B16 melanoma which is refractory to anguidine and other trichothecenes.

N-(Phosphonacetyl)-L-aspartic acid (PALA), a rationally synthesized inhibitor of aspartate transcarbamylase was tested against a spectrum of experimental tumors. It proved to be a solid tumor-specific antimetabolite with a high degree of activity against six of the eight solid tumors tested (Lewis lung carcinoma, B16 melanoma, Glioma 26, M5076 ovarian carcinoma, C3H mammary carcinoma, colon carcinoma 26) but was inactive in four leukemias. The basis for this selective effect on solid tumors was shown to be related to the levels of the target enzyme which are lower in the solid tumors. The toxic and antineoplastic effects of PALA *in vivo* could be reversed by carbamyl aspartate, thus proving that the effects of this drug are due to inhibition of aspartate transcarbamylase.

Anguidine, bruceantin, homoharringtonine and bouvardin are protein synthesis inhibitors. The relationship between their cytotoxicity and the extent and duration of protein synthesis inhibition was examined. It has been found that protein synthesis must be completely inhibited longer than 4 hours in order to obtain greater than a 3 log cell kill. These studies indicate the importance of maintaining effective plasma levels after administration of these agents to tumor-bearing animals and probably also to humans.

An effort was made to evalute new drugs under development for clinical trial against a panel of three colon carcinoma xenografts. The drugs tested have reproducible activity against murine tumors, and their antitumor effects are due to a variety of mechanisms. The tumor xenografts failed to respond to the drugs tested, indicating that colon carcinoma xenografts may be useful models for evaluating the potential utility of new drugs for treating human colon carcinoma.

A program was initiated to develop a 2450 MHz microwave apparatus for the controlled local application of hyperthermia to murine tumors. The project was directed at perfecting applicators and servo mechanisms for heating solid tumors weighing as much as 1 gm. Preliminary studies with murine mammary carcinomas and colon carcinoma xenografts showed that an oncolytic effect could be achieved, but temperature nonuniformity resulted in variability of response. Current efforts are directed toward development of a system which will uniformly heat peripheral and visceral tumors.

Further in vitro and in vivo studies on the folic acid antagonists have been performed. In vitro cloning studies of normal mouse bone marrow have shown that reversal of methotrexate toxicity can be achieved with Ca-leucovorin and nucleosides. Toxicity development and reversal is a function of both time of exposure and concentration of MTX and is unique for different tissues. The reversal of toxicity is a function of concentration of both Ca-leucovorin and nucleosides. Ca-leucovorin provides a competitive reversal with respect to MTX, whereas the nucleosides require an absolute minimum value before reversal occurs and they become toxic at values approaching 10^{-4} Molar. In vivo studies in mice revealed that reversal of MTX toxicity can be achieved with Ca-leucovorin and nucleosides. With leucovorin the ratio of MTX concentration to leucovorin concentration is critical. Data also suggest that the time of exposure to MTX may determine the time at which successful rescue with leucovorin must commence. MTX at low concentration ($\sim 2 \times 10^{-8}$ M) is toxic to mice because of a thymineless state in the small intestine, and this toxicity can be reversed with thymidine alone if the proper blood thymidine concentration is achieved ($\sim 10^{-5}$ M). At a higher MTX concentration (2×10^{-7} M) reversal of toxicity requires a purine source such as inosine in addition to thymidine.

Pharmacokinetic, biochemical and antitumor evaluation of polymeric methotrexate derivatives are under evaluation. MTX has been bound covalently by ester and amide linkages to carriers such as poly-l-lysine, carboxymethylcellulose, and divinylether-malic anhydride (DIVEMA) copolymer. These polymers vary with regard to those structural features, (e.g., ionic charge and molecular weight) which are most likely to affect cellular uptake. The DIVEMA polymer also has established antitumor properties of its own, probably due to its immune stimulating properties. Studies to date have concentrated on the DIVEMA-MTX polymer. This copolymer shows in vitro inhibition of dihydrofolate reductase similar to free MTX, in contrast to the cellulose-MTX polymer which is about 1000-fold less potent. DIVEMA-MTX was as effective as an equivalent dose (5 mg/kg) of MTX in inhibiting ^{3}H-UdR incorporation into DNA of femur and small intestine of mice. A considerable increase in life span of mice bearing leukemia L1210 was found at low MTX-equivalent doses. Further studies with solid tumors are planned, particularly with those resistant to MTX treatment such as the Lewis lung tumor.

Pharmacologic studies in laboratory animals were continued for four anticancer agents [chlorozotocin, spirohydantoin mustard, 3-deazauridine and AMSA (NSC-141549)] and studies with three new drugs (pyrazalo-imidazole, aminothiadiazole and quinolinium dibromide) have been initiated. Studies on the pharmacokinetics of gallium in humans were continued and the pharmacokinetics of cis-diamminedichloroplatinum (DDP) in humans are under investigation in conjunction with Phase II clinical trials. Preliminary results with DDP indicate that plasma levels peak at 20-40 μM, platinum and then decline in two distinct phases. The initial rapid $T_{1/2}$ of 50-70 minutes is followed by a long second $T_{1/2}$, measured in days. Three weeks after the last infusion of DDP, platinum levels are still detectable in the blood (1.5-4.0 μM). These chronically elevated platinum levels accent the importance of remaining alert to signs of heavy metal toxicity in patients on long term therapy with DDP.

Studies on the disposition of chlorozotocin-[glucose-1-^{14}C] and [-ethyl-2-^{14}C] in rats were continued and were extended to the dog. After administration to rats (10 or 20 mg/kg, iv), the renal clearance of the glucose-^{14}C label was rapid, with 75% of the dose appearing in urine within 6 hr, and only 4% in bile. In contrast, 37% of the ethyl-^{14}C label was excreted in urine within 6 hr and 40% in bile. However, only a small proportion of the ethyl label appeared in the feces of rats with no biliary cannula, suggesting that enterohepatic circulation of the species carrying the chloroethyl moiety takes place. Urinary excretion of both labels in the dog was similar to that in the rat, but slightly slower. The levels of radioactivity in plasma declined in a biphasic manner. The half-life for the initial distribution phase (14 min) and that for the unbound radioactivity for the second phase (146 min) were independent of dose, position of label and animal species. Binding markedly reduces the rate of decline of total radioactivity in plasma after the distribution phase and accounts for differences in the half-lives for the two labeled species. Tissue distribution studies in rats at 6 hr and dogs at 24 hr showed significantly higher concentrations of ethyl-^{14}C than glucose-^{14}C in the liver and kidney. Levels of ethyl-^{14}C were also significantly higher in the CSF of dogs for the first two hours after administration of the drug. Much of this data supports the view that cleavage of the chlorozotocin molecule occurs between its glucose and chloroethyl moieties.

Data on the disposition of [^{14}C]-quinolinium dibromide was obtained in rats. By 96 hours after an ip dose (2.6 mg/kg) of the drug, only 16% of the radioactivity had been excreted in urine and 24% in the feces; 14% and 18% remained in the kidneys and liver, respectively. Tissue specific activity was highest in the kidney. Clinical protocols involving a schedule of daily treatments should take into account the prolonged tissue retention of the drug. The slow clearance of radioactivity is probably due to binding of quinolinium dibromide to cellular components, since the majority of ^{14}C in kidney and liver homogenates is precipitated by 10% trichloroacetic acid. Quinolinium dibromide binds tightly to calf thymus DNA as demonstrated by simultaneous elution with DNA from a Sephadex G200 column.

The pharmacologic disposition of ^{14}C-aminothiadiazole (AT) was studied in rats given an iv dose of 12.5, 25 or 50 mg/kg. Radioactivity was distributed evenly to all tissues (including brain), and tissue to plasma ratios of approximately 1.0 were found for most tissues. However, in small intestine, liver, kidney and adrenals, ratios greater than 2.0 were obtained four hours after drug administration. Plasma concentrations of drug remained nearly constant during the first 6 hours after iv administration, despite minimal in vitro binding of drug to plasma proteins. Levels of tissue and plasma radioactivity were proportional to dose. Urinary excretion was the major route of elimination, with more than 70% of the administered dose eliminated in 24 hours. Chromatographic analysis of urine and plasma revealed no metabolites. There was no evidence of metabolites in homogenates of any tissue except for small intestine and stomach, where a single minor metabolite was found. The relatively high levels of unchanged AT found in the brain (brain to plasma ratio of 1.0), suggest that this drug may have potential utility in treating brain tumors.

A mathematical analysis of intra-arterial (intracarotid) and intravenous drug distribution to the brain (the assumed site of a tumor) and the bone marrow and splanchnic tissue (the presumed sites of toxicity), have led to the following conclusions. The desirable characteristics of a drug for brain tumor chemotherapy, regardless of the route of administration, are a high affinity for only the tumor and an ability to exchange readily with only the tumor. The intracarotid infusion of a particular drug will always yield somewhat higher brain tissue concentrations than a similar intravenous infusion. This intracarotid advantage (increased brain delivery and decreased marrow and splanchnic tissue distribution) is especially significant for a drug with a high affinity and/or blood-tissue exchange rate for the tumor only. If a drug causes significant systemic toxicity and is fairly rapidly metabolized ($t_{1/2} \leq 10$ min), then an intra-arterial infusion of this compound would be superior, in terms of drug distribution, to an intravenous one. The administration of any drug into an artery which perfuses the tumor at a low rate produces much higher tumor and lower marrow and splanchnic tissue concentrations than a matching intravenous infusion. In the next few months aminothiadiazole and spirohydantoin mustard (SHM) will be given to animals by the intracarotid and intravenous route for assessment of the relative advantages of each route for treatment of CNS tumors.

SHM is an antitumor agent with activity against intracranially implanted murine ependymoblastoma. Although this activity suggests that the drug crosses the blood-brain barrier, levels of SHM in the CSF or brain have not been determined. This study was undertaken to quantitatively measure levels of the free, unaltered drug in the CSF and blood of dogs after intravenous administration of SHM. A gas chromatographic-mass spectrometric method developed to measure ng quantities of SHM was used. Following intravenous administration to dogs (4 mg/kg), the levels of SHM in the blood declined in a biphasic manner. The initial disappearance of SHM from the blood was rapid with a half-life of 1.78 and 1.85 min for 2 different dogs. The second phase was slower, but still fast, with a half-life of about 11 min. SHM did pass the blood-brain barrier reaching a maximum level in the CSF at approximately 10 min. Although actual concentrations in the CSF were low, all of the compound present was the parent compound which contains the active alkylating moiety. SHM labeled with ^{14}C in the hydantoin and chloroethyl moieties is now available and studies are in progress to determine the pharmacological and metabolic fate of SHM in rats and dogs. Emphasis is being placed on the measurement of drug penetration and binding in the brain.

Disposition studies of C^{14}-pyrazalo-imidazole in rats were performed using iv doses of 100 and 500 mg/kg. Plasma radioactivity disappearance curves were dose related, with $T_{1/2}$ values of 120 min and 360 min at doses of 100 mg/kg and 500 mg/kg, respectively. The prolonged $T_{1/2}$ in vivo may explain the finding that the optimal therapeutic schedule in leukemic mice is one dose every four or eight days. Six hours after administration, radioactivity was distributed to all tissues (including brain) with highest concentrations in stomach > liver > kidney. Significantly high levels were also found in thyroid, perhaps accounting for the increased follicular activity of the thyroid epithelia reported in toxicology studies. There was only a minimal amount of radioactivity appearing in the bile. Chromatographic studies are in progress to detect and identify metabolites in urine, plasma and tissues.

Conjugation with glucuronic acid frequently plays an essential role in the terminal metabolism of drugs and endogenous compounds, including steroids, thyroid hormone and bilirubin. Results to date suggest that multiple forms of glucuronyl transferase are present in hepatic microsomes and probably other tissues. Further studies of these enzymes may aid in the design of cytotoxic antitumor agents as well as steroids with antitumor activity but minimal host toxicity.

An antitumor agent carried selectively by lymphatic channels would be likely to have therapeutic advantages in treating lymphoid malignancies and tumors which metastasize by lymphatics. Studies were undertaken to examine the extent to which clinically effective antitumor agents were absorbed and distributed via the lymphatics. The intestinal absorption of most of the clinically-effective antitumor agents tested to date takes place primarily via the portal route rather than by the intestinal lymphatic system. Following the intraduodenal administration of radioactive CCNU, methyl-CCNU, guanazole, dibromomannitol, diethylstilbestrol, prednisolone, dexamethasone, 6-mercaptopurine, cytosine arabinoside, and cyclophosphamide, 5% or less of the administered radioactivity was recovered in 24-hour thoracic duct lymph. The only antitumor agent tested so far which appears to be selectively absorbed by the intestinal lymphatics is o,p-DDD. Following the administration of this compound in aqueous solution, 12.2% of the administered radioactivity was recovered in thoracic duct lymph, and this figure was increased to 26.1% when the compound was administered as a suspension in corn oil. In addition to assessing the extent to which antitumor agents are absorbed and distributed by the lymphatics, it is hoped to define the chemical and/or structural characteristics required for the selective uptake of compounds by lymphatics. In achieving this objective, it would be possible to design antitumor agents for selective lymphatic absorption.

Studies designed to elucidate the molecular basis of cytotoxicity of several new anticancer agents were performed. The interaction of AMSA with DNA and its effects on nucleic acid polymerizing enzymes were examined, and equilibrium dialysis and spectrophotometric techniques revealed that its binding to DNA is similar to that of other aminoacridines. Fluorescence studies indicated interactions between AMSA and the purine and pyrimidine rings of DNA suggestive of intercalation. DNA melting studies showed that AMSA stabilized the DNA double helix but not to the same extent as other aminoacridines. AMSA concentrations of 200 to 400 μg/ml were required to inhibit mouse embryo DNA polymerase and AMV reverse transcriptase. Enzyme inhibition was greatest with templates containing only adenine-thymine base pairs. The relatively high concentrations of AMSA required for inhibition of these enzymes *in vitro* as compared to concentrations necessary for *in vitro* cytotoxicity or for *in vivo* antitumor activity suggest that the interaction with DNA alone might not fully explain its marked chemotherapeutic effectiveness.

Studies on the pharmacologic disposition of AMSA demonstrated that it was selectively localized in the liver following oral administration, with reduced exposure to other tissues. The *in vivo* detoxification of AMSA by liver involves direct interaction with glutathione-S-transferase, non-enzymatic chemical reaction with glutathione, and active transport of metabolites into the bile. Total cellular content of glutathione-S-transferase appears to be directly related to the extent of differentiation. The enzyme constitutes 5-10% of normal liver protein and is absent or barely detectable in fetal liver, monkey hepatocellular carcinoma or L1210 leukemia. Thus, it was anticipated that orally administered AMSA might prove to be selectively toxic against liver metastases or hepatocellular carcinoma because of impaired detoxification mechanisms in these tumors. To test this hypothesis, L1210 cells were injected iv into mice to mimic liver metastases. The mice were treated orally 5 days later, and the livers bioassayed 24 hours after treatment. A dose of 500 mg/kg (that was non-toxic and inactive against ip implanted L1210) reduced the population of L1210 cells from 2.5×10^6 cells/liver in untreated mice to undetectable levels in 6 out of 10 treated mice. Studies are presently underway to assess the effectiveness of AMSA administered orally to monkeys bearing chemically induced hepatocellular carcinomas. Effectiveness of treatment is being followed by X-ray scanning, α-fetoprotein levels and liver biopsy in 5 monkeys.

A series of bis-quinaldine derivatives, consisting of two substituted quinaldine moieties coupled by an alkyl bridge, have been synthesized. These compounds possess many of the structural attributes of double or polyintercalating agents. Such a molecule can bind at two positions in the same DNA molecule or bind to two different strands of DNA. These compounds were found to be extremely toxic *in vitro*, to inhibit DNA-dependent RNA polymerase, to stabilize DNA as determined by Tm measurements and to exhibit *in vivo* antitumor activity against P388 leukemia (T/C of 110 - 186%).

A comparison of the metabolism of deazauridine by normal and leukemic cells *in vivo* revealed no qualitative differences between the different cell types. However, there was a quantitative difference between normal and malignant cells in the formation of deazaUTP. Thus, at 1/2, 1, 2 and 4 hours after a therapeutic dose (300 mg/kg, ip) of deazauridine in mice, concentrations of deazaUTP in L1210 cells were approximately 100-fold higher than in normal cells. DeazaUTP inhibits CTP synthetase (thought to be the site of action of deazauridine) with a K_I of 5.3×10^{-6} M. Thus inhibition of CTP formation would be expected to be transient in normal tissues but prolonged in L1210 cells. Deazauridine was found to be a substrate for uridine kinase isolated from normal mouse tissues and L1210 cells. There was no correlation between tissue levels of deazauridine nucleotides and levels of uridine kinase, an enzyme that could possibly function to "trap" nucleosides intracellularly as the nucleotide form. This lack of correlation supports a recent hypothesis that cellular transport of uridine (and hence, deazauridine) plays a greater role in determining cellular concentrations of uridine nucleosides and nucleotides than does phosphorylation.

Studies have been initiated to define the mechanism by which some chemicals induce neoplastic cells to redifferentiate and to assess the ability of nucleophilic agents to protect against the carcinogenic effects of alkylating antitumor agents and other chemical carcinogens. Studies to date indicate that polar compounds such as DMSO can induce the neoplastic cell to "redifferentiate" and loose some of its malignant characteristics.

New antitumor agents and those in clinical use are being evaluated for embryotoxic and cytogenetic effects. A comparative study of a group of plant-derived antitumor agents (vincristine, maytansine, colchicine, VP 16-213 and VM-26) has been completed. At equimolar doses, vincristine possesses approximately 2-fold greater embryolethal and teratogenic activity than does maytansine, and these effects are most pronounced when treatment is given on day 7 of gestation. Less marked embryotoxicity was encountered with colchicine, VP 16-213 and VM-26. At equimolar doses, VM-26 possessed the most marked embryotoxic activity, regardless of the day of gestation (day 6, 7 or 8) on which the compounds were given. A strict correlation between embryotoxic and cytogenetic effects was not found in this study. The most severe cytogenetic damage, comprising both structural and numerical chromosome aberrations, was found in embryos exposed to the drugs on day 8 of gestation. Vincristine, the most teratogenic of the compounds tested, did not cause marked chromosomal damage in embryonic tissue; in contrast, VP 16-213 induced a variety of aberrations in embryonic chromosomes, although it is a relatively weak teratogen. These results suggest that, under the conditions employed, the embryotoxicity of the compounds in this series may not be associated with embryonic cytogenetic damage.

Studies are in progress on the embryotoxicity of PALA and 6-azauridine, two inhibitors of *de novo* pyrimidine nucleotide biosynthesis. Daily treatment with PALA for 5 days during early gestation resulted in 100% intrauterine deaths in litters exposed to a dose (3 mg/kg) approximately 100-fold lower than that found optimal for increasing the survival of mice inoculated with B16 melanoma and Lewis lung carcinoma. When pregnant mice were given a single injection of PALA on day 7 of gestation, it was observed that the slope of the dose-response curve was remarkably steep. Thus, at 10 mg/kg, the intrauterine mortality rate was 24% and a low incidence of fetal malformation was found, whereas at 12.5 mg/kg, 90% of the exposed fetuses were killed, and 80% of the survivors were malformed. No embryos survived a dose of PALA of 25 mg/kg given on day 7 or 8 of gestation. Preliminary results with 6-azauridine suggest that it too has potent embryotoxic activity. When given on days 7-11 of gestation at 37 mg/kg, a 90% incidence of intrauterine death resulted, and all of the surviving fetuses were severely malformed.

Polycyclic hydrocarbons such as benzo[a]pyrene, methylcholanthrene and dimethylbenzanthracene can be metabolized in the ovaries of rats and mice by a cytochrome P-450 dependent monooxygenase. The mouse ovarian monooxygenase (aryl hydrocarbon hydroxylase) is inducible by polycyclic hydrocarbon treatment. The susceptibility of the ovarian enzyme to induction by polycyclic hydrocarbons is inherited as an autosomal dominant trait, as is the case for the enzyme in liver, lung and other organs. Furthermore, the degree of damage to primordial oocytes induced by polycyclic hydrocarbons is closely correlated with the activity of ovarian aryl hydrocarbon hydroxylase. This is of importance, since the first step in the initiation of granulosa cell tumors in mice is the destruction of primordial oocytes.

The availability of a large breeding and experimental monkey colony (600 animals representing 4 species) continues to provide this laboratory and other cooperating units with a unique resource for comparative pharmacologic, toxicologic, and biochemical studies in non-human primates. Various antitumor agents, including adriamycin, methylnitrosourea (MNU), procarbazine, melphalan and Imuran are being evaluated for carcinogenic activity in long-term studies in monkeys. A total of 55 monkeys have been treated with procarbazine for periods ranging from 1 to 11 years. To date, ten monkeys (18%) have developed a malignancy, and 6 of the 10 neoplasms were acute myelogenous leukemias. None of the monkeys treated with adriamycin, melphalan, or Imuran have developed tumors as yet, but studies with these drugs have only been underway for 24 months or less. MNU is under clinical trials in the USSR and has been reported to be effective in the treatment of lung cancer. We have evaluated its carcinogenic potential in three species of non-human primates. A total of 43 monkeys have received MNU for periods up to 96 months. Five of the 13 monkeys (38%) necropsied thus far have had squamous cell carcinoma (SCA) of the oro-pharynx and/or esophagus, and 12 of the 13 (92%) have had some type of upper digestive tract lesion such as atrophy or dyskeratosis of the esophageal mucosa and esophagitis. All of the animals receiving total doses of MNU exceeding 50 gm developed SCAs after approximately 5 years of exposure. Five other chemicals under study have induced tumors in three species of Old World monkeys. These include 3 nitrosamines: [diethylnitrosamine (DENA), 1-nitrosopiperidine (PIP) and dipropylnitrosamine (DPNA)], MAM-acetate, and aflatoxin B_1. The nitrosamines and aflatoxin B_1 have induced primary liver cell carcinomas, and MAM-acetate has induced both primary liver cell carcinomas and carcinomas of the kidney. Alpha-fetoprotein (AFP) levels in sera of monkeys with biopsy-proven liver tumors were measured using radioimmunoassay. The wide range of values (100,000-fold variation) was analogous to that seen in patients with hepatocellular carcinoma. Monkeys with DENA-induced neoplasms had significantly higher serum levels of AFP than those with tumors induced by 1-nitrosopiperidine, MAM-acetate or aflatoxin B_1. Serial determinations of AFP levels in monkeys with liver tumors demonstrated that the values tended to plateau after reaching a level characteristic for that tumor.

The induction of hepatic and lung cytochrome P-450 dependent monooxygenases by 3-methylcholanthrene has been studied in male rhesus monkeys. Hepatic microsomes were also studied for their ability to activate 2-acetylaminofluorene to its mutagenic form. Hepatic n-hydroxylation of 2-acetylaminofluorene is increased 6-7 fold following 3-methylcholanthrene treatment; a similar increase is observed for aryl hydrocarbon hydroxylase in both liver and lung. The hepatic cytochrome P-450 content is increased by 50% and the Soret maximum in the CO-hemoprotein complex is shifted to the blue by 2 nm after 3-methylcholanthrene induction. Electrophoresis of hepatic microsomes from 3-methylcholanthrene treated monkeys revealed an increase in a band corresponding to a polypeptide with a molecular weight of 54,000. Activation of 2-acetylaminofluorene to its mutagenic form by liver microsomes from untreated rhesus monkeys is very low, but is increased 40 fold after 3-methylcholanthrene treatment. No effect is observed on the activation of N-hydroxy-2-acetylaminofluorene by liver microsomes following 3-methylcholanthrene treatment. These studies may explain the lack of carcinogenic activity of 2-acetylaminofluorene in rhesus monkeys.

The effects of intrathecal heparin upon the acute and chronic phases of the increases in CSF hydrostatic pressure and absorption resistance associated with subarachnoid hemorrhage were evaluated in the rhesus monkey. The results indicate that the administration of heparin into the cerebral subarachnoid space abolished the changes associated with subarachnoid bleeding during the recovery phase.

In addition to the basic laboratory research effort, each senior investigator shares the responsibility for the administration and supervision of one or more contracts with outside research institutions. The contracts are primarily concerned with the development of new tumor systems in which to evaluate antitumor agents; studies on resistance and mechanisms of resistance to new antitumor agents; the study of pharmacological disposition of antitumor drugs and their metabolites in humans and laboratory animals; the development of pharmacokinetic mathematical models; and the study of chemical carcinogens and biological markers for detection of cancer and assessment of cancer treatment. Approximately 10 new antitumor agents are studied intensively each year, reflecting the interest of the Developmental Therapeutics Program and the Division of Cancer Treatment in developing clinically effective antitumor drugs.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 03500-13 LCHP

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

The Distribution of Drugs Between Blood, Brain, and Cerebrospinal Fluid

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
7.0	4.5	2.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Intracarotid drug infusions for the treatment of brain tumors appear to offer a significant advantage in terms of optimal distribution when the drug has either a high affinity for the tumor only, a high rate of blood-tumor exchange, or a rapid rate of metabolism or when carotid blood flow is low. Chronic alterations in body tissue and fluid levels of vitamin A in rhesus monkeys were found to produce no detectable changes in CSF pressure and absorption and in brain cellular and capillary permeability. Our results indicate that the CSF pressure and absorption changes which occur when blood is injected into the subarachnoid space (subarachnoid hemorrhage) are substanially alleviated when the injected blood is heparinized. We have evidence in the rhesus monkey that albumin is moved from brain to blood by a special transport system or "pump". A critical examination of the Oldendorf BUI technique indicated that this procedure for measuring blood-brain barrier permeability has blood flow, sampling time, and reference material limitations. We have also found that the regulation of brain solute levels is only slightly controlled by the sink action of the CSF. Finally we demonstrated that brain edema occurs in two species of fish.

Continuation of PHS-6040

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

OTHER:	D. G. Gomez	Asst. Professor, Cornell University
	H. F. Cserr	Asst. Professor, Brown University
	J. A. Kessler	Resident, Cornell University

Project Description:

Objectives:

The objectives of this project are the quantification of the transport of various materials of physiological and pharmacological importance between blood, neural tissue, and cerebrospinal fluid (CSF) and the examination of the effects of various pharmacological agents and pathological conditions upon the transport and metabolism of essential materials, e.g. simple sugars, within the blood-brain-CSF system. The characterization of the transport properties of the blood-brain-CSF system, the study of volume regulation of the central nervous system (CNS) and CSF, and the development of methods for obtaining more effective concentrations of drugs in the CNS are the goals of this work.

Methods Employed:

The method of ventriculocisternal perfusion of various radioactive materials and subsequent brain tissue sampling has been utilized in the rhesus monkey and dogfish shark for measurements of brain capillary and cellular permeability and of brain extracellular movements of solutes and fluid. Studies of brain capillary permeability by intracarotid injection of a bolus of 3 to 5 radioactively labeled materials and rapid (1-90 seconds after the injection) sampling of brain tissue have been performed in rats. A method of examining blood-tissue exchange using 4 or 5 gamma-emitting isotopes (a vascular space marker, a labeled protein such as albumin, a blood-flow indicator, and two tracer materials of unknown transfer properties) has been developed and used in studies on normal rats and rhesus monkeys. Along with this, a technique for obtaining numerous samples of circulating blood in rapid succession by means of a 3-way valve catheter apparatus which connects the femoral artery and vein has been developed and tested in rats and rhesus monkeys. A quick, reproducible method of inducing cerebral edema with a freeze lesion has been perfected in mice, rats, and dogfish sharks. By using two different labeled forms of the same compound, a way of comparing the distribution of that material either at two different time points or by two different routes of administration (e.g. intravenous and intraperitoneal) in the same animal has been devised. Finally, computer modeling has been used to study drug distribution between blood, CNS, and CSF following simulated intravenous, intra-arterial, and intraventricular administration.

Major Findings:

From our mathematical analysis of intra-arterial (intracarotid) and intravenous drug distribution to the brain (the assumed site of a tumor) and the bone marrow and splanchnic tissue (the presumed sites of toxicity), we have come to the following conclusions. The desirable characteristics of a drug for brain tumor chemotherapy, regardless of the route of administration, are a high affinity for only the tumor and an ability to exchange readily with only the tumor. A drug which has a high affinity and exchange rate with some or all of the other tissues of the body does not provide such a marked chemotherapeutic advantage. The intracarotid infusion of a particular drug will always yield somewhat higher brain tissue concentrations than a similar intravenous infusion. This intracarotid advantage (increased brain delivery and decreased marrow and splanchnic tissue distribution) is especially significant for a drug with a high affinity and/or blood-tissue exchange rate for the tumor only. If a drug causes significant systemic toxicity and is fairly rapidly metabolized ($t_{1/2} \leq 10$ min), then an intra-arterial infusion of this compound would be advantageous, in terms of drug distribution, to an intravenous one. The administration of any drug into an artery which perfuses the tumor at a low rate produces much higher tumor and lower marrow and splanchnic tissue concentrations than a matching intravenous infusion.

In view of the effects of vitamin A on the central nervous system (increased intracranial pressure in man with hypervitaminosis A, known clinically as pseudotumor cerebri, and elevated CSF outflow resistance and pressure in calves with hypovitaminosis A) and the potential usages of high dose vitamin A and vitamin A analogues in certain solid tumor chemotherapy protocols, a chronic study of the action of this vitamin on neurological function was undertaken with three groups -- high, low, and normal vitamin A diets -- of rhesus monkeys. During the 50 month study period, alterations in the blood and tissue levels of the vitamin were found; however no detectable changes in CSF production, pressure, and absorption and in brain cellular and capillary permeability were observed. It is concluded that high and low dietary levels of vitamin A do not produce marked neurological changes in the rhesus monkey.

Using a rhesus monkey model, an assessment was made of the effects of intrathecal heparin upon the acute and chronic phases of the increases in CSF hydrostatic pressure and absorption resistance associated with subarachnoid hemorrhage. The results indicate that the administration of heparin into the cerebral subarachnoid space caused smaller pressure and absorption changes in the acute phase and abolished the chronic phase changes in these two CSF hydrodynamic parameters associated with subarachnoid bleeding.

From our ventriculocisternal perfusion studies, we have obtained evidence that brain and CSF levels of albumin are kept low by means of a "pump" which transfers albumin from brain extracellular fluid to blood. The half-time of this transport process is around 30 minutes, and thus, the depth of tracer albumin penetration from CSF into brain tissue is greatly limited by the clearance of this protein across the capillaries of the brain.

We have also extended our ventriculocisternal perfusion studies to several neurotransmitter metabolites, homovanillic acid (HVA) and 5-hydroxyindoleacetic acid (5-HIAA), and to two inorganic anions, iodide and bromide. In work with the rhesus monkey, we have found no evidence of special transport systems which mediate the flow of these acids and ions between blood and brain and the clearance of these materials from brain tissue, as has been suggested by other investigators in other species. In earlier work from our laboratory we demonstrated that HVA and HIAA are fairly readily transported from spinal cord tissue to blood by a simple diffusion-like mechanism which was unaffected by probenecid (spinal cord capillary exchange $t_{1/2}$'s $\approxeq$ 20 min). Although the same transfer process, diffusion, may be involved in the movements of these organic acids across brain capillaries, the rates appear to be much slower ($t_{1/2}$'s $\geq$ 60 min).

A number of investigators have hypothesized that the limited uptake or accumulation of certain materials by CNS tissue is due as much to the "sink" action of the CSF as to the tightness of CNS capillaries, the blood-brain barrier phenomona. For the sink hypothesis of CSF function, it is postulated that materials which enter brain extracellular fluid (ECF) by leakage, either from blood into the ECF via the capillaries or from brain cells into the ECF, can be cleared from the ECF by diffusion through this fluid into the CSF and subsequent "washing away" by entrainment in the circulating CSF. We have examined this postulation in the rhesus monkey and the dogfish shark (a creature that has as much CSF as brain tissue and, thus, has a large CSF "sink" potential) with materials such as urea, sodium, inulin, and albumin. Our experimental and computer modeled data show that the effectiveness of the CSF sink depends on the capillary permeability and the tissue diffusivity of each individual molecular species and that this route of solute clearance from the CNS is of very limited importance for most of the materials we have studied, including ones as diverse in size and characteristics as sodium and albumin. Special transport systems sited at the capillaries and physically tight capillary walls seem to be the general way of regulating brain fluid composition.

A critical evaluation of Oldendorf's brain uptake index (BUI) technique, a new method for quickly determining relative brain capillary permeabilities, was made in rats. Our data shows that the BUI depends upon the rate of cerebral blood flow, the sampling time, the injected volume of the test solution, and the reference tracer chosen for the study. Furthermore the use of this experimental approach in systems which are altered by drug treatments, experimental manipulations, or disease conditions is not acceptable unless a preliminary set of studies which establishes the basic experimental parameters for the perturbed system, such as the sampling time and the reference marker, are done first.

Preliminary work in the dogfish shark with 3-O-Methyl-D-Glucose, a non-metabolizable transport analogue of D-glucose, suggests that the distribution of this compound between blood and brain, blood and CSF, and blood and vitreous humor occurs by a carrier-mediated process. Such special transport systems for glucose and other simple sugars are necessary since the blood-brain, blood-CSF, and blood-vitreous barriers block the passive diffusional exchanges of such essential metabolites between these fluid and tissue compartments.

Experimental work from our laboratory has indicated that the blood-brain barrier not only restricts the transfer of solutes between blood and brain but also limits the diffusional and osmotic flow of water in this system. Osmotic and hydrostatic pressure changes which induce significant volume changes in other tissues have little or no effect upon CNS volume because these two factors, the slow exchange of fluid and the impermeability of blood-brain barrier to the principal body fluid solutes, namely sodium, potassium, and chloride, buffer fluid shifts between blood, brain, and CSF. In certain pathological conditions, such as cerebral trauma and brain tumors, the blood-brain barrier is broken down. Our theoretical and computer analyses of this condition indicate that disruption of the blood-brain barrier will lead to significant changes in brain volume and to brain edema. Generally the production of brain edema constitutes a greater clinical problem than the primary lesion itself.

As a continuation of the preceding studies in CNS volume regulation, we have begun to experimentally examine the mechanism of cerebral edema development and resolution in several animal species. Our data indicate that a freeze lesion causes cerebral edema in the dogfish shark and the goldfish, two animals which have very low brain vascular pressures. In the dogfish the permeability of the brain capillaries to sodium and albumin were not appreciably altered at a time when brain enlargement was maximal. Work with a rat brain edema model has also indicated a similar dissociation between brain swelling and blood-brain barrier tightness. These observations suggest that cerebral edema may be caused, in some instances, by osmotic forces within the tissue rather than vascular hydrostatic pressure.

Significance to Biomedical Research and the Program of the Institute:

The exchange of material between blood and CNS tissue and blood and CSF is quite different than blood-tissue and blood-fluid exchanges in most other organs of the body. This is not only because of the presence of transport "barriers" between these compartments but also because of the unique relationship between the CNS and CSF. In normal animals these systems work to maintain a very precise condition of homeostasis and volume balance within the brain and spinal cord. There are several drawbacks which result from these complex regulatory arrangements. Diseases (e.g. brain tumors), surgery, drug treatments, radiation therapy, and trauma can disturb these systems in very specific or very general ways and lead to complications which are greater in impact upon the CNS than the original lesion. Furthermore, the delivery of pharmaceutical agents for the treatment of various nervous system disorders to sites within the CNS are often blocked by these transport barriers. Finally, the toxic metabolic products of many pathological processes are trapped within the neural parenchyma by these regulatory systems and subsequently render further tissue damage. Basic knowledge of the transport of fluids, ions, metabolites, drugs, and other materials between blood, brain, and CSF in normal and altered conditions is essential to the understanding of the activities and functions of the CNS and the treatment of nervous system diseases.

With our laboratory's studies, we seek to gain fundamental information on the basic nature of these transport systems, the optimal way of administering drugs for the treatment of CNS diseases, the structural characteristics which will facilitate drug uptake by neural tissue, and the abberations in these homeostatic mechanism which are caused by various CNS and CSF lesions and sundry treatment procedures (e.g. surgery, radiation, and chemotherapy). In addition the experimental techniques which are developed by our group for the quantification of blood-brain-CSF transport processes are and will be used by other laboratories. In summary, the type of work performed in our laboratory is useful and significant to the neurologist, neurosurgeon, neuro-oncologist, and neuropathologist as well as the neurophysiologist, neurochemist, and neuropharmacologist.

Proposed Course:

1. Continue our normal animal studies of the transport of various physiological and pharmacological materials between blood, brain, and CSF.

2. Develop new and more precise ways of quantitating brain blood flow, blood-brain barrier permeability, and brain cell permeability.

3. Study further the flow of solutes and water between blood, brain, and CSF and their respective roles in CNS volume regulation in normal animals and in experimental models of brain edema, hydrocephalus, and brain tumors.

4. Examine the effects of high and low dietary levels of vitamin A and vitamin A analogues (currently referred to as "retinoids") on CSF production, flow, and absorption and on CNS capillary and cellular permeability.

5. Seek for more effective routes of drug delivery to the CNS and for antitumor drugs which will be readily taken up by brain tissue because of their structural similarities to known blood-brain barrier penetrating compounds.

6. Investigate, whenever appropriate brain tumor models and fresh animal specimens are available, the transport characteristics of the sundry types of CNS and meningeal tumors.

7. Study the clearance mechanisms for proteins, peptides, acid metabolites, and inorganic ions from CNS tissue.

8. To develop, in collaboration with several clinical groups, a non-invasive way to gauge CSF levels and clearances of intrathecally administered drugs, such as methotrexate, by means of gamma camera and EMI scans and various radiopharmaceutical agents.

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 03502-14 LCHP

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)
Folic Acid Antagonists in Experimental Tumors and Normal Tissues of Mice

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	D. S. Zaharko	Head, Pharmacokin. & Pharmacodyn. Sect.	LCP	NCI
OTHER:	W. P. Fung	Staff Fellow	LCP	NCI
	K. H. Yang	Visiting Fellow	LCP	NCI
	M. Przybylski	Visiting Fellow	LCP	NCI
	R. L. Dedrick	Chemical Engineer	BEIB	DRS
	R. J. Lutz	Chemical Engineer	BEIB	DRS

COOPERATING UNITS (if any)
Biomedical Engineering and Instrumentation Branch, DRS

LAB/BRANCH
Laboratory of Chemical Pharmacology

SECTION
Pharmacokinetics and Pharmacodynamics Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS: 3.0	PROFESSIONAL: 3.0	OTHER: None

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Constant infusions of MTX into mice have been conducted in order to provide us further understanding on organ specificity with respect to rate of inhibition and rate of recovery of certain biochemical pathways and cell growth. Reversal of inhibition and selectivity of this reversal by nucleosides and folinic acid has also been investigated.

Transport kinetics of MTX and thymidine in vivo in normal tissues and tumors is being studied by use of pharmacokinetic modeling.

Initial investigations have begun with MTX linked to synthetic polymers. Testing of their selective toxic response in vivo is being conducted. In vitro studies with MTX resistant and sensitive tumor lines are being started.

PHS-6040
(Rev. 10-76)

Project Description:

Studies in mice with mini-infusion devices developed through a cooperative effort with Drs. Dedrick and Lutz of BEIB are continuing. Constant infusions of MTX alone are being brought to a conclusion. We are currently developing an infusion device that can be inserted subcutaneously for the use of thymidine. Such investigations we believe will provide further insight eventually into whether or not increased selectivity of antimetabolite type antitumor agents can be achieved with concomitant administration of endogenous salvage metabolites, such as thymidine.

To date we have shown with in vitro cloning studies of normal mouse bone marrow that reversal of methotrexate toxicity can be achieved with Ca-leucovorin and nucleosides. Toxicity development and reversal is a function of both time of exposure and concentration of MTX. This characteristic is unique for different tissues. The reversal of the toxicity is a function of both concentration of Ca-leucovorin and nucleosides. Ca-leucovorin provides a competitive reversal with respect to MTX whereas the nucleosides require an absolute minimum value before reversal occurs and begin to show toxicity at values approaching 10^{-4} Molar.

In vivo studies in mice approached with the same concepts arrived at from in vitro cloning studies indicate that reversal of MTX toxicity can be achieved with Ca-leucovorin and nucleosides. With leucovorin the ratio of MTX concentration to leucovorin concentration is critical. Data also suggest that the time of exposure to MTX may determine the time at which successful rescue with leucovorin must commence. Infusions with MTX, leucovorin and thymidine indicated that MTX at low concentration ($\sim 2 \times 10^{-8}$ M) essentially is toxic to mice because of a thymineless state in the small intestine and this toxicity can be reversed with thymidine alone if the proper blood thymidine concentration is achieved ($\sim 10^{-5}$ M). At a higher MTX concentration (2×10^{-7} M) reversal of toxicit requires a purine source such as inosine in addition to thymidine.

A synthesis of the information gathered from studies with the murine Lewis lung tumor - pharmacokinetic and biochemical - indicate that the resistance of this tumor to MTX is due to a combination of several factors: 1) the enzyme DHFR is six times higher than in the small intestine 2) the growth fraction is about one quarter of that in the small intestine 3) there is a resistance to membrane transport of MTX which is currently being quantitated by a pharmacokinetic analysis in terms of a transport Km and Vmax. A peripheral finding as a result of ^{3}H-MTX studies in the Lewis lung tumor was that degradation products of MTX in mouse blood after about three hours represent a major part of the radioactivity. This opens up a new aspect of potential cause of MTX toxicity in vivo by metabolic products of MTX with lesser but nonetheless sufficient binding to DHFR to manifest toxicity.

Pharmacokinetic, biochemical and antitumor evaluation of polymeric methotrexate derivatives are under evaluation. MTX has been bound covalently by ester and amide linkages to carriers, such as poly-l-lysine, carboxymethylcellulose, and divinylether-malic anhydride (DIVEMA) copolymer. These polymers encompass different structural features, such as ionic charge, and molecular weight which are most likely to affect cellular uptake. The DIVEMA polymer has additionally, established antitumor properties of its own, which by past investigations, have been shown to be primarily through its immune stimulating properties.

Studies to date have concentrated on the DIVEMA-MTX polymer. This copolymer shows in vitro inhibition of dihydrofolate reductase similar to free MTX in contrast to the cellulose-MTX polymer which is about three orders of magnitude less potent in vitro. DIVEMA-MTX given in vivo to mice exhibited similar inhibition of ^{3}H-UdR into DNA as that caused by MTX at an equivalent dose of 5 mg/kg in femur and small intestine, with perhaps later recovery. Considerable increase in life span was found in L1210 tumor bearing mice at low MTX-equivalent doses. High toxicity appears to be limiting when equivalent doses and schedules that are optimal for the free drug MTX are used. Further studies are planned with solid tumors particularly those resistant to MTX treatment, such as the Lewis lung tumor. Other cooperative studies with investigators at Roswell Park Memorial Institute with additional solid tumor lines are being considered.

To assess the pharmacokinetic effect of polymer fixation of MTX, the plasma kinetics of MTX are being studied. Investigations are also being carried out to determine whether spontaneous or enzymatic hydrolysis of MTX from the polymer occurs. Chromatographic studies, ultra filtration, in vitro enzyme inhibition and the fate of ^{14}C-labeled MTX-copolymer in vivo are currently being planned.

Publications:

1. Zaharko, D. S. and Dedrick, R. L.: Antifolates: In Vivo Kinetic Considerations. Cancer Treatment Reports. 61(4): in press, July, 1977.

2. Zaharko, D. S.: Chemokinetics. Environmental Health Perspectives. in press, 1977.

3. Zaharko, D. S., Fung, W. P. and Yang, K. H.: Relative Biochemical Aspects of Low and High Doses of MTX in Mice. Cancer Research. 37: Vol. 37, in press, 1977.

4. Pinedo, H. M., Zaharko, D. S. and Dedrick, R. L.: Device for Constant Sc Infusion of Methotrexate: Plásma Results in Mice. Cancer Treatment Reports. 60: 889-893, July, 1976.

5. Pinedo, H. M., Zaharko, D. S., Bull, J. and Chabner, B. A.: The Relative Contribution of Drug Concentration and Duration of Exposure to Mouse Bone Marrow Toxictiy during Continous MTX Infusion. Cancer Res. 37: 445-450, Feb., 1977.

6. Pinedo, H. M., Chabner, B. A., Zaharko, D. S. and Bull, J. M.: Evidence for Early Recuritment of Granulacyte Precursors During High-Dose MTX Infusions in Mice. Blood. 48: 301-307, Aug., 1976.

7. Pinedo, H. M., Zaharko, D. S., Bull, J. M. and Chabner, B. A.: The Reversal of MTX Cytotoxicity to Mouse Bone Marrow Cells by Leucovorin and Nucleosides. Cancer Research. 36: 4418-4424, Dec., 1976.

Pharmacokinetics and Pharmacodynamics Section
Contracts

Contract No. N01-CM-67064

Principal Investigator: Dr. Daniel S. Zaharko

Mario Negri Institute of Pharmacological Research

$227,712

Project Description:

This contract has the purpose of investigating the relationship between drug kinetics and drug effect with the adriamycin analogue N-trifluoro-acetyladriamycin-14-valerate (AD32). Initial studies have been conducted to compare this analogue with adriamycin and daunomycin in L1210 tumor bearing mice. Effects such as increase in life span, immunosuppression, and colony formation of bone marrow cells have been looked at. Initial studies on tissue distribution and metabolism of AD32 have been conducted.

Further work on identification of metabolites and tissue distribution will be conducted. The cytotoxic effect of metabolites _in vitro_ will be checked. Correlation of tissue pharmacokinetic effects with biological effects such as inhibition of DNA synthesis and immunosuppression will be conducted.

These type of data will be valuable in understanding the mechanism of action of this drug and should be of value to program in determining the emphasis to be placed on this drug in clinical trials.

This contract was incrementally funded for a three year period. It was recently reviewed and recommended for further support fot its second year.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 03505-14 LCHP

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)
Potential Embryotoxic, Carcinogenic and other Adverse Effects of Anticancer Agents

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	S. M. Sieber-Fabro	Pharmacologist	LCP	NCI
OTHER:	R. H. Adamson	Chief, Lab.of Chem.Pharm.	LCP	NCI
	J. Whang-Peng	Head, Cyto. Oncology Sect.	MO	NCI

COOPERATING UNITS (if any)
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LAB/BRANCH
Laboratory of Chemical Pharmacology

SECTION
Pharmacology and Experimental Therapeutics Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	0.5	0.5

CHECK APPROPRIATE BOX(ES)
☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

New antitumor agents, as well as clinically established drugs are being evaluated for embryotoxic, cytogenetic and carcinogenic effects. A comparative study of the embryotoxic and cytogenetic effects in mice of a series of plant-derived antitumor agents (vincristine, maytansine, colchicine, VP 16-213 and VM-26) has been completed. Studies have been initiated on the embryotoxic and cytogenetic effects of two adenosine deaminase inhibitors (2'-deoxycoformycin and EHNA), and of two inhibitors of de novo pyridmide nucleotide biosynthesis (N-[phosphonacetyl]-L-aspartic acid and 6-azauridine). The carcinogenic activity of various clinically effective antitumor and/or immunosuppressive agents, including procarbazine, N-methylnitrosourea, melphalan, azathioprine and adriamycin, is under evaluation in long-term studies in monkeys. A survey of second malignancies developing in treated cancer patients, initiated several years ago, is continuously being updated using data from published reports and from several centers and hospitals.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives:

This project is designed to obtain data on various aspects of the toxicity of antitumor agents, including their embryotoxic, cytogenetic and carcinogenic effects in rodents, monkeys and in the human. Specifically, the objectives are:

To evaluate in mice the embryotoxic and cytogenetic effects of various classes of antineoplastic agents. These agents include selected plant-derived drugs with clinical as well as experimental antitumor activity, adenosine deaminase inhibitors, and inhibitors of de novo pyrimidine nucleotide biosynthesis. The goal of such studies is to define differences in teratogenic potency among groups of agents with similar mechanisms of antitumor action and antitumor spectra. It is anticipated that such differences can be exploited in clinical situations in which women of child-bearing age require antitumor and/or immunosuppressive therapy. Studies are also being directed at defining the duration of the cell cycle in those embryonic tissues most susceptible to the teratogenic effects of cytotoxic chemicals. Such studies will provide information as to the relationship between gestational age and cell cycle duration, the ability of cytotoxic agents to alter embryonic cell cycle kinetics, and the consequences of such an alteration. In addition, studies are underway to follow the time course of embryonic drug-induced cytogenetic damage and the extent to which embryonic tissue is capable of repairing such damage.

To determine in mice whether treatment of animals prior to mating with some clinically effective antitumor agents alone (methotrexate, actinomycin D) or in combination (MOPP regimen) has an adverse effect on their offspring. The ultimate goal of this study is to evaluate the ability of the treated animals to reproduce, and to assess the viability of their offspring. Particular emphasis is given to the fertility of subsequent generations of these animals and the frequency with which they develop tumors.

To evaluate in monkeys the carcinogenic potential of various clinically useful antitumor agents administered singly and in combination with other agents and/or irradiation. In addition, the effects of such treatment on other functions, such as the immune system and reproduction, is under examination. A significant proportion of monkeys receiving prolonged procarbazine treatment have developed a malignancy, more than half of which have been acute leukemia (see below). The surviving monkeys presently receiving procarbazine are considered to be at high risk of developing acute leukemia, and detailed hematologic and cytogenetic studies are currently being carried out in this group of animals. The goal of these studies in the procarbazine-treated monkeys is to determine whether a "preleukemic" condition exists in chemically-induced acute leukemia, and if so, to characterize it.

To monitor the current medical literature and the NCI patient data in order to tabulate and characterize adverse effects of antineoplastic agents in man. Particular emphasis is being given to embryotoxic, mutagenic and carcinogenic effects arising from treatment with clinically effective antitumor agents.

Major Findings:

Embryotoxicity of Antitumor Agents

A comparative study of the embryotoxic and cytogenetic effects of a group of plant-derived antitumor agents (vincristine, maytansine, colchicine, VP 16-213 and VM-26) has been completed. At equimolar doses, vincristine possesses approximately 2-fold greater embryolethal and teratogenic activity than does maytansine, and these effects are most pronounced when treatment is given on day 7 of gestation. Less marked embryotoxicity was encountered with colchicine, VP 16-213 and VM-26. At equimolar doses, VM-26 possessed the most marked embryotoxic activity, regardless of the day of gestation (day 6, 7 or 8) on which the compounds were given. A strict correlation between embryotoxic and cytogenetic effects was not found in this study. The most severe cytogenetic damage, comprising both structural and numerical chromosome aberrations, was found in embryos exposed to the drugs on day 8 of gestation. Vincristine, the most teratogenic of the compounds tested, did not cause marked chromosomal damage in embryonic tissue; in contrast, VP 16-213 induced a variety of aberrations in embryonic chromosomes, although it is a relatively weak teratogen. These results suggest that, under the conditions employed, the embryotoxicity of the compounds in this series may not be associated with embryonic cytogenetic damage.

Studies on the embryotoxic and cytogenetic effects of two inhibitors of the enzyme adenosine deaminase, 2'-DCF and EHNA, are underway. 2'-DCF administered daily for 5 days during early gestation does not possess marked embryotoxic activity at the dose (0.25 mg/kg) found optimal for enhancement of the antitumor activity of adenosine analogs against experimental animal tumors. However, dose-related increases in the incidence of intrauterine mortality, intrauterine growth retardation, and fetal malformations were noted at higher (0.5-2.0 mg/kg) dose levels of this drug. Results with EHNA, although preliminary, suggest that it also is without striking embryotoxic activity when given at doses (6-25 mg/kg) which potentiate the antitumor action of adenosine analogs.

Studies are in progress on the embryotoxicity of PALA and 6-azauridine, two inhibitors of de novo pyrimidine nucleotide biosynthesis. Daily treatment with PALA for 5 days during early gestation resulted in 100% intrauterine deaths in litters exposed to a dose (3 mg/kg) approximately 100-fold lower than that found optimal for increasing the survival of mice inoculated with B16 melanoma and Lewis lung carcinoma. When pregnant mice were given a single injection of PALA on day 7 of gestation, it was observed that the slope of the dose-response curve was remarkably steep. Thus, at 10 mg/kg, the intrauterine mortality rate was 24% and a low incidence of fetal malformations was found, whereas at 12.5 mg/kg, 90% of the exposed fetuses embryos were killed, and 80% of the survivors were malformed. No embryos survived a dose of PALA of 25 mg/kg given on day 7 or 8 of gestation. Preliminary results with 6-azauridine suggest that it too has potent embryotoxic activity. When given on days 7-11 of gestation at 37 mg/kg, a 90% incidence of intrauterine death resulted, and all of the surviving fetuses were severely malformed.

Evaluation of antitumor agents for carcinogenic activity in monkeys

From our survey of second tumors in treated cancer patients, we are accumulating evidence that Hodgkin's disease patients receiving treatment with the MOPP regimen, one component of which is procarbazine, are at increased risk of developing AML. Although a causal relationship between cytotoxic drug therapy and the appearance of AML in these patients has not yet been established, there is ample evidence that procarbazine is a potent carcinogen in mice and rats. In addition, the carcinogenic potential of procarbazine in 3 species of non-human primates is being evaluated. A total of 55 monkeys have received procarbazine for periods up to 11 years. Ten of the 39 monkeys (26%) necropsied thus far have had malignant neoplasms, six of which were AML. Two monkeys developed osteogenic sarcomas, and single cases of hemangiosarcoma and lymphocytic lymphoma were found. The average total dose of procarbazine received by the monkeys developing malignancies was 37.9 gm, the average duration of procarbazine treatment was 76 months. The 16 surviving monkeys in this study may represent a population at high risk for developing AML. For this reason, they are being utilized in detailed hematologic and cytogenetic studies in order to determine whether a "preleukemic" phase is detectable prior to the development of frank drug-induced leukemia. In addition, a number of the toxic effects of procarbazine seen clinically were also noted in the monkeys which have been necropsied, including vomiting and myelosuppression. Its most striking toxic effect, however, was on the reproductive system of the males. The majority of the adult males necropsied to date have had testicular atrophy with complete aplasia of the germinal epithelium.

N-methylnitrosourea (MNU) is under clinical trials in the USSR and has been reported to be effective in the treatment of lung cancer. We have evaluated its carcinogenic potential in three species of non-human primates. A total of 43 monkeys have received MNU for periods up to 96 months. Five of the 13 monkeys (38%) necropsied thus far have had squamous cell carcinoma (SCA) of the oro-pharynx and/or esophagus, and 12 of the 13 (92%) have had some type of upper digestive tract lesion such as atrophy or dyskeratosis of the esophageal mucosa and esophagitis. All of the animals receiving total doses of MNU exceeding 50 gm developed SCAs after approximately 5 years of exposure. Many parallels were noted between the esophageal SCA observed in the present series of monkeys and human esophageal carcinoma, including the clinical manifestations of the tumor, its complications, its radiographic appearance and its morphology. The MNU-induced lesions of the oro-pharynx and esophagus of primates may therefore be a valuable model for the study of human esophageal carcinoma.

Melphalan is currently being tested as adjuvant therapy in patients with breast and ovarian cancer. However, there is some evidence that patients receiving long-term treatment with melphalan for multiple myeloma or ovarian cancer may be at increased risk of developing AML. Although a causal relationship between melphalan treatment and the development of AML in these patients has not yet been established, the carcinogenic activity of this agent has been demonstrated in mice and rats. The carcinogenic potential of melphalan in non-human primates is therefore under study. Twenty monkeys are currently being treated by the oral route with melphalan (0.1 mg/kg) daily, 5 days a week. To date, none of these monkeys have developed a malignancy; however, this study has been underway for less than 2 years.

Publications:

1. Adamson, R. H. and Sieber, S. M.: Antineoplastic agents as potential carcinogens. In Hiatt, H. H., Watson, J. D. and Winsten, J. A. (Eds.): Cold Spring Harbor Conferences on Cell Proliferation, Vol. 4, Origins of Human Cancer. (In press).

2. Sieber, S. M. and Adamson, R. H.: Some comments on the potential carcinogenicity of the clinically useful antitumor agents. In Cahan, W. G., Schottenfeld, D. and Moertel, C. G. (Eds.): Proceedings of the International Workshop on Multiple Primary Cancers. New York, October 7-8, 1976, (In press).

3. Sieber, S. M.: The action of antitumor agents: a double-edged sword? Medical and Pediatric Oncology. (In press).

4. Adamson, R. H., Krowlikowski, F. J., Correa, P., Sieber, S. M. and Dalgard, D. W.: Carcinogenicity of 1-methyl-1-nitrosourea (MNU) in non-human primates. J. Natl. Cancer Inst. (In press).

5. Sieber, S. M., Mead, J. A. R. and Adamson, R. H.: Pharmacology of antitumor agents from higher plants. Cancer Treatment Rep. 60: 1127-1140, 1976.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 03506-14 LCHP

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Pharmacology and Disposition of Antitumor Drugs

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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COOPERATING UNITS (if any)

None

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
4.0	2.5	1.5

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Pharmacologic disposition studies in laboratory animals were continued (see previous report) for four agents [chlorozotocin (NSC-178248), spirohydantoin mustard (NSC-172112), 3-deazauridine (NSC-126849) and AMSA (NSC-141549)]. Studies were initiated for three additional agents in laboratory animals [pyrazalo-imidazole (NSC-51143), aminothiadiazole (NSC-4728) and quinolinium dibromide (NSC-176319)]. These studies were designed to obtain information that will be of use by clinicians in the design of clinical trials. Such information is essential for a successful clinical evaluation of the agent. A study of the pharmacokinetics of cis dichlorodiamino-platinum (NSC-119875) in humans was initiated in conjunction with Phase II clinical trials, and a special study on the distribution of this agent in the monkey CSF was begun. An in vitro study on the properties of rat liver glucuronyl transferase was performed.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives:

To study the metabolic fate of experimental antitumor agents prior to clinical trial and to ascertain whether their metabolites are active in various tumor systems.

To study the pharmacology of antitumor drugs of current interest in clinical chemotherapy in order to design better therapeutic regimens.

To study the clinical pharmacology of new agents in phase I clinical trial.

To evaluate the usefulness of new tumor systems in experimental chemotherapy.

To design newer anticancer drugs by rational approaches.

Methods Employed:

Usual pharmacologic, biochemical, analytical, and immunologic techniques.

Major Findings:

Disposition of Spirohydantoin Mustard (SHM), NSC-172112

SHM is an antitumor agent with activity against intracranially implanted murine ependymoblastoma. Although this activity suggests that the drug is crossing the blood-brain barrier, levels of SHM in the CSF or brain had not been determined. This study was undertaken to quantitatively measure levels of the free, unaltered drug in the CSF and blood of dogs after intravenous administration of SHM. A gas-chromatographic-mass-spectrometric method developed to measure ng quantities of SHM was used. Following intravenous administration to dogs (4 mg/kg), the levels of SHM in the blood declined in a biphasic manner. The initial disappearance of SHM from the blood was rapid with a half-life of 1.78 and 1.85 min for 2 different dogs. The second phase was slower, but still fast, with a half-life of about 11 min. SHM did pass the blood-brain barrier reaching a maximum level in the CSF at approximately 10 min. Although actual concentrations in the CSF were low, the drug being measured is the administered parent compound which contains the active antitumor alkylating moiety. SHM labeled with ^{14}C in the hydantoin and chloroethyl moieties is now available and studies are in progress to determine the pharmacological and metabolic fate of SHM in rats and dogs. Emphasis is being placed on the measurement of drug penetration and binding in the brain.

Studies with Quinolinium dibromide, NSC-176319

Preliminary data on the disposition of [^{14}C]-quinolinium dibromide was obtained in rats. Following administration of the drug (2.6 mg/kg, ip), only 16% of the radioactivity was excreted in the urine within 96 hr and 24% in the feces with 14 and 18% remaining in the kidneys and liver, respectively. Tissue specific activity was highest in the kidney. Any clinical protocol involving daily scheduling should consider the prolonged elevated concentrations of the drug in the tissues. The slow clearance of radioactivity from the animal is probably due to binding of quinolinium dibromide to cellular components as the majority of ^{14}C in kidney and liver homogenates is precipitated by 10% trichloroacetic acid. Quinolinium dibromide binds tightly to calf thymus DNA as demonstrated by simultaneous elution with DNA from a Sephadex G200 column. Also, the absorption spectrum of quinolinium dibromide is altered by the interaction with DNA.

Disposition of Chlorozotocin, NSC-178248

Studies on the disposition of chlorozotocin-[glucose-1-^{14}C] and [-ethyl-2-^{14}C] in rats were continued and were extended to the dog. After administration to rats (10 or 20 mg/kg, iv), the renal clearance of the glucose -^{14}C label was rapid with 75% of the dose appearing in urine within 6 hr, and only 4% in bile. In contrast, 37% of the ethyl-^{14}C label was excreted in urine within 6 hr and 40% in bile. However, only a small proportion of the ethyl label appeared in the feces of rats with no biliary cannula suggesting an enterohepatic circulation of the species carrying the chloroethyl moiety. Urinary excretion of both labels in the dog was similar to that in the rat, but slightly slower. The levels of radioactivity in plasma declined in a biphasic manner. The half-life for the initial distribution phase (14 min) and that for the unbound radioactivity for the second phase (146 min) were independent of dose, position of label and animal species. Binding markedly reduced the rate of decline of total radioactivity in plasma after the distribution phase and accounts for differences in the half-lives for the two labeled species. Tissue distribution studies in rats at 6 hr and dogs at 24 hr showed significantly higher concentrations of ethyl-^{14}C than glucose-^{14}C in the liver and kidney. Levels of ethyl-^{14}C were also significantly higher in the CSF of dogs for the first two hours after administration of the drug. Much of this data supports the view that cleavage of the chlorozotocin molecule occurs between its glucose and chloroethyl moieties.

Disposition of 3-Deazauridine, NSC-126849

Studies on the pharmacologic disposition of deazauridine in normal and leukemic mice were extended (see previous annual report). Concentrations of deazauridine in plasma, tissues and urine following ip administration of doses of 75, 150 and 300 mg/kg were found to be directly proportional to dose. Deazauridine is phosphorylated by all normal and leukemic cells *in vivo* to the corresponding nucleoside mono-, di-, and triphosphate forms as quantitated by ion exchange column chromatography. There was no detectable incorporation into the DNA or RNA of normal or leukemic cells in vivo. Neither the pyridine nucleus nor the ribose moiety underwent metabolic alteration other than phosphorylation of the 5'-OH group and the glycosidic linkage was not disrupted. Cleavage of the glycosidic linkage by intestinal bacteria has limited the oral usefulness of other pyrimidine nucleoside analogues (e.g. azauridine). Experiments were performed to determine if the stability of the glycosidic linkage of deazauridine could be exploited to permit oral administration. C^{14}-Deazauridine labelled in either the ribose moiety (1'-C^{14}-deazauridine) or the pyridine moiety (6-C^{14}-deazauridine) was administered orally and the radioactivity in tissues, plasma, urine and feces determined in parallel experiments. Chromatographic analyses demonstrated identical disposition patterns for the two labelled species indicating that no disruption of the glycosidic linkage had occurred. Furthermore, a dose of 300 mg/kg (the optimal therapeutic ip dose) was nearly completely absorbed (>90% of the administered dose) and the tissue distribution at 60 min was nearly identical to that obtained after ip administration of the same dose. Urinary excretion was the primary route of elimination with either route of administration. Plasma levels after oral administration remained constant at 1 to 5 x 10^{-4}M from 15 min to 6 hours and dropped to 5 x 10^{-5}M at 24 hours. In contrast, ip administration resulted in a biphasic plasma disappearance curve that ranged from 1 x 10^{-3}M at 15 min to 1 x 10^{-5}M at 7 hours. Cytotoxicity experiments using L1210 in vitro indicated that concentrations of deazauridine greater than 1 x 10^{-6}M completely inhibited growth. These results indicate the use of deazauridine orally to achieve sustained plasma levels of cytotoxic concentrations of deazauridine with identical disposition patterns as that achieved with parenteral administration.

Disposition of Aminothiadiazole (AT), NSC-4728

C^{14}-AT was used to study the pharmacologic disposition of AT in rats after iv administration of doses of 12.5, 25 and 50 mg/kg. Radioactivity was distributed evenly to all tissues (including brain) and tissue to plasma ratios of approximately 1.0 were found for most tissues except small intestine, liver, kidney and adrenals where values of greater than 2.0 were obtained four hours after administration. Plasma concentrations of drug remained nearly constant during the first 6 hours after iv administration; although the drug exhibited only minimal binding to plasma proteins or albumin in vitro. Tissue and plasma levels of radioactivity determined at various doses were found to be proportional to dose. Urinary excretion is the major route of elimination with more than 70% of the administered dose eliminated in 24 hours. Less than 2% of the dose was eliminated in the bile after 6 hours. Chromatographic analysis of urine, plasma and tissue homogenates revealed no detectable metabolites except in homogenates of small intestine and stomach where one spot containing less than 10% of tissue radioactivity was detected in one of the eight solvent systems employed. One of the most interesting aspects of AT derived from the disposition data is the high levels of unchanged drug found in the brain (brain to plasma ratio of 1.0), suggesting a potential use of this drug against brain tumors.

Disposition of 4'-(9-Acridinylamino)-methanesulfon-m-anisidide (AMSA), NSC-141549

In an earlier study (see previous annual report) of the pharmacologic disposition of AMSA in rodents following iv administration, AMSA was found to be especially vulnerable to nucleophilic attack by alkylthiols resulting in displacement of 4-amino-3-methoxymethanesulfonanidide and the formation of the corresponding 9-alkyl-thioether of acridine. Such a non-enzymatic attack by low molecular weight thiols (e.g. glutathione) results in a product that is eliminated in the urine and bile; whereas, interaction with protein thiol groups results in prolonged retention of the acridine nucleus in plasma and tissues. Following iv administration, AMSA was rapidly removed from plasma by the liver and the metabolites of AMSA actively transported into the bile. Based on the biliary transport data we theorized that selective localization of AMSA in the liver might be achieved by oral administration since compounds absorbed from the GI tract must pass through the liver before reaching the systemic circulation. In this manner, reduced exposure of other tissues to the drug might be attained. AMSA was found to be well absorbed from the GI tract and the metabolic fate of AMSA the same as that shown previously following iv administration. The major route of elimination after oral administration is biliary and the major radioactive biliary metabolite (accounting for 90-95% of total biliary radioactivity) possesses identical chromatographic properties as the thiolysis product of AMSA and glutathione formed in vitro. Although the ultimate metabolic fate of AMSA is the same whether administered orally or iv, it is of particular significance that the tissue distribution of AMSA was altered by the route of administration. Concentrations of AMSA and AMSA metabolites were similar in liver and small intestine with either route of administration, but were greatly reduced in plasma and all other organs following oral administration. A chemical assay for AMSA was developed based on our finding that hydroxide ion attacks the 9-carbon of AMSA forming acridone, one of most fluorescent molecules known. The fluorescent assay technique allows measurement of AMSA concentrations as low as 1×10^{-9} M. The technique will be employed in studies of the clinical pharmacology of AMSA.

Disposition of Pyrazalo-Imidazole, NSC-51143

Disposition studies of C^{14}-pyrazalo-imidazole in rats were performed at doses of 100 and 500 mg/kg, iv. Plasma radioactivity disappearance curves were dose dependent with $T_{1/2}$ values of 120 min and 360 min at doses of 100 mg/kg and 500 mg/kg, respectively. The prolonged $T_{1/2}$ in vivo may explain the optimal therapeutic schedule in leukemic mice being once every four or eight days. Six hours after administration, radioactivity was generally distributed to all tissues (including brain) with highest concentrations in stomach > liver > kidney with significantly high levels in thyroid, accounting, perhaps, for the increased follicular activity of the thyroid epithelia reported in toxicology studies. There was only a minimal amount of radioactivity appearing in the bile. Chromatographic studies are in progress to detect and identify metabolites in urine, plasma and tissues.

The Pharmacokinetics of cis-Dichlorodiamino-platinum, NSC 119875 (DDP) in Patients undergoing Phase II Clinical Trials

The technique of atomic absorption spectroscopy is being used to determine blood and urine levels of platinum in patients receiving DDP for treatment of breast and ovarian carcinoma. It is hoped these studies will provide information on the relationship of clinical toxicity and clinical antitumor effects to the blood levels and excretory patterns of the drug. Although the study is ongoing, the available data show that patients are obtaining peak plasma levels of 20-40 μM platinum which then fall in two distinct phases. The initial rapid fall ($T_{1/2}$ of 50-70 minutes) is followed by a long second phase ($T_{1/2}$ measured in days). The rapid phase decline lowers blood levels to 35-50% of peak values within 1-2 hours and then blood values slowly decline thereafter. Three weeks after the most recent infusion of DDP, platinum levels are still detectable in the blood (1.5-4.0 μM). These chronically elevated platinum levels accent the importance of observing for heavy metal toxicity in patients on long term therapy with DDP.

The Distribution of cis-Dichlorodiamino-platinum, NSC-119875 (DDP) in Monkey CSF

Clinical studies with DDP have revealed several instances of apparent seizures following infusion of DDP. Monkeys were used to study the ability of DDP to enter the CSF following intravenous injection. The monkeys have a surgically implanted Ommays reservoir which allows multiple sampling of the CSF. Monkeys were given DDP at doses of 1.5 and 3.0 mg/kg, iv. Blood and CSF were sampled following injection and analyzed for platinum using atomic absorption spectroscopy. CSF levels of platinum were detectable, with peak levels occurring 30 minutes after the iv injection. The CSF peak levels were 0.3 and 0.6 μM platinum after 1.5 and 3.0 mg/kg doses, respectively. CSF platinum levels were undetectable (less than 50 nM) at 3 hours post infusion. The peak CSF levels were 1-2% of the peak blood levels. These results provide a physical basis for the clinically observed seizures and may lead to further work on metal coordination complexes as agents for CNS malignancy.

Studies on Glucuronyl Transferase

Conjugation with glucuronic acid frequently plays an essential role in the terminal metabolism of both drugs and endogenous compounds including steroids, thyroid hormone and bilirubin. Important among the pathways for the metabolism of chlorpromazine are its hydroxylation to pharmacologically active metabolites which are metabolically inactivated by conjugation with glucuronic acid.

We have previously observed that the glucuronidation of 7-hydroxychlorpromazine (CPOH) by microsomes of rat liver is stimulated by CPOH itself at concentrations greater than 3 x 10^{-4}M and by Triton X-100, a nonionic detergent. Subsequent studies showed that both the magnitude of the stimulation of CPOH conjugation by Triton and the profile of the Triton concentration - activity curves were dependent upon the concentration of CPOH. The maximal, four-fold stimulation of the conjugation of CPOH (1.6 x 10^{-4}M) was observed with Triton 0.025%, v/v. With high concentrations of CPOH (7 x 10^{-4}M) the maximal stimulation, relative activity 1.3 (control 1.0), was found with Triton 0.005%. CPOH thus reduces both the magnitude of the stimulation and the concentration of Triton required for the maximal activation of glucuronyl transferase. Since increasing the concentration of Triton itself would cause identical effects, these findings suggest that CPOH is surface active and that concentrations of the substrate greater than 3 x 10^{-4}M stimulate its conjugation by a Triton-like action which exposes additional active sites of glucuronyl transferase.

It appears that the interaction of inhibitors with glucuronyl transferase is best characterized by its variability. For example, depending upon the substrate, SKF-525A, the classical inhibitor of the microsomal mixed function oxidases, either inhibits glucuronidation in a competitive or non-competitive manner or has no effect on conjugation. Although the rate of conjugation of CPOH was maximal at pH 7.0 a second peak with about 60% of the maximal activity was observed at pH 8.5. Interestingly, when CPOH was incubated with SKF-525A (1 x 10^{-3}M) a 70% inhibition of glucuronidation was observed at pH 7.0, but activity at pH 8.5 was unchanged. In view of the formidable body of indirect evidence which is presently available to support the hypothesis that multiple forms of glucuronyl transferase are present in hepatic microsomes, these findings suggest that the conjugation of CPOH may be catalyzed by several forms of glucuronyl transferase which differ markedly in their sensitivity to inhibition by SKF-525A.

Publications:

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2. Dingell, J. B. and Sossi, N.: Studies on the glucuronidation of 7-hydroxychlorpromazine *in vitro*. Drug Metabolism and Disposition. (in press).

3. Cysyk, R. L., Shoemaker, D. D., Ayers, O. C. and Adamson, R. H.: Oral absorption and selection tissue localization of 4'-(9-acridinylamino)-methanesulfon-m-anisidide. Pharmacology. (in press).

4. Cysyk, R. L., Shoemaker, D. D. and Adamson, R. H.: The Pharmacologic Disposition of 4'-(9-acridinylamino)-methanesulfon-m-anisidide in Mice and Rats. Drug Metabolism and Disposition. (in press).

5. Cysyk, R. L., Gormley, P. E., D'Anna, M. E. and Adamson, R. H.: The Pharmacologic Disposition of 3-Deazauridine in Mice. Drug Metabolism and Disposition. (in press).

6. Cysyk, R. L. and Adamson, R. H.: The Pharmacologic Disposition of Inosine Dialdehyde (NSC-118994) in Mice, Rats, Dogs and Monkeys. Cancer Treatment Rep. 60: 555-562, 1976.

7. Plowman, J., Cysyk, R. L. and Adamson, R. H.: The disposition of Coralyne Sulphoacetate in Rodents. Xenobiotica. 6: 281-294, 1976.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 03509-14 LCHP

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Carcinogenesis, Chemotherapy and Biological Markers in Non-Human Primates

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Various chemicals are being administered to various species of non-human primates to obtain comparative data on the response of non-human primates and rodents to known carcinogens and to materials suspected to be carcinogenic for humans. In addition, it is hoped that biological markers which will aid in the diagnosis of preneoplastic change as well as for frank neoplasia will be developed. Thus far seven chemicals have induced tumors in Old World monkeys; these are diethylnitrosamine, dipropylnitrosamine, 1-nitrosopiperidine, methylnitrosourea, methylazoxymethanol acetate, aflatoxin B_1 and procarbazine. The serum levels of alpha-fetoprotein by radioimmunoassay in monkeys with biopsy-proven liver tumor were scattered over a wide range of 100,000 fold variation which is analogous to the range seen in human hepatocellular carcinoma.

Studies have been undertaken to try to prevent hepatic carcinogenesis in non-human primates by pretreatment with various nucleophiles such as cysteamine and cysteine before administration of the carcinogen.

Project Description:

Objectives:

This project is designed to obtain comparative data on the response of non-human primates and rodents to known carcinogens and to materials suspected to be carcinogenic for humans and to develop model primate tumor systems which may be predictable for therapy for humans and for development of biological markers. Specifically the objectives are:

To study the effects of compounds, generally of potential carcinogenic activity in newborn primates and to determine if the newborn primate, like the rodent, will be highly susceptible to carcinogenesis.

To evaluate the carcinogenic potential and long-term effects of anticancer drugs which are being used clinically for long-term remissions and for diffuse collagen disorders.

To develop model non-human primate tumor systems.

To try to prevent carcinogenesis or reverse it in non-human primates by the use of nucleophiles, immunostimulation or administration of antitumor drugs before histological evidence of tumor.

To obtain data on which to base a comparison of the response of non-human primates and rodents to materials known or suspected to be carcinogenic to man, and thereby to evaluate the relative merits of rodents and non-human primates in predicting for man.

To use normal and tumor-bearing non-human primates for studying the pharmacological, toxicological, and chemotherapeutic properties of various anticancer, antiviral, and antimicrobial agents.

To use the various tumors and animals with premalignant changes and with tumors for biochemical and immunological studies.

To develop biological markers which will aid in the diagnosis of preneoplastic changes as well as for existing neoplasia.

Methods Employed:

Usual pharmacologic, biochemical, clinical, anatomical pathologic, cytological, and immunologic techniques as well as breeding and nursing care of infant monkeys and special dietary regimens.

Major Findings:

General Information:

The present colony consisting of 600 animals is comprised of basically four species: Macaca mulatta, Macaca fascicularis, Cercopithecus aethiops, and Galago crassicaudatus. Of these 520 are experimental; the remaining 80 are adult breeders which supply the newborns for experimental study.

Previous annual reports have contained detailed information concerning the management and maintenance procedures employed, the hematological, biochemical and diagnostic parameters monitored and information concerning the methods utilized in treating the newborns. These procedures have not been significantly altered and are, therefore, not included in this report.

No major disease problems have been encountered during this period.

Compounds Not Carcinogenic in Non-Human Primates:

The compounds which have not shown carcinogenic potential thus far in Old World monkeys after treating at least 10 monkeys for 5 years and an additional holding period of 3 years are: N-2-fluorenylacetamide, low density polyethylene plastic, DDT, saccharin, cyclamate, N-N'-dimethyl-p-phenylazoanaline, and N,N'-dimethyl-p-(m-tolylazo)-analine.

Antitumor Agents Under Evaluation for Carcinogenic Potential:

Antitumor agents which are under evaluation for carcinogenic potential include procarbazine, methylnitrosourea, adriamycin, L-phenylalanine mustard, and imuran. Thus far, procarbazine and methylnitrosourea have induced tumors in Old World monkeys. Procarbazine has induced acute myelogenous leukemia, osteogenic sarcoma, lymphoma and hemangiosarcoma while methylnitrosourea induced tumors of the upper digestive tract. Procarbazine has induced neoplasms in 18 percent of treated monkeys after an average latent period of 6-1/2 years and methylnitrosourea induced squamous cell carcinoma of the oro-pharynx in 11 percent of monkeys receiving this compound for an average of 6 years.

Other Compounds Inducing Tumors:

In addition to procarbazine and methylnitrosourea 5 other comounds have induced tumors in monkeys. These are diethylnitrosamine, dipropylnitrosamine, 1-nitrosopiperidine, aflatoxin B_1 and methylazoxymethanol acetate (MAM acetate). Aflatoxin B_1 has induced hemangioendothelial sarcoma of the pancreas and liver as well as carcinoma of the liver. MAM acetate has induced carcinoma of the kidney in two monkeys and hepatic cell carcinoma in four monkeys. The three nitrosamines have induced primary liver carcinomas in approximately 120 monkeys. Experiments are in progress to try to establish if there is a dose response and/or a threshold carcinogenic dose for the hepatocarcinogen diethylnitrosamine (DENA). Five groups of monkeys are receiving biweekly intraperitoneal injections of DENA at doses of 1, 5, 10, 20 or 40 mg/kg. Thus far monkeys have developed tumors after administration of 10, 20 or 40 mg/kg with the latent period for development of tumor increasing as the mg/kg dose decreases. In addition, a total dose of DENA between 1.2 and 1.5 gm seems to be required for tumor induction regardless of the biweekly dose.

Prevention of DENA Carcinogenesis

Several nucleophiles are being administered to monkeys prior to dosing with the hepatocarcinogen DENA. It is hypothesized that if exogenously administered nucleophiles are available at the site where the carcinogens exert their electrophilic attack that carcinogenesis may be prevented. Such an approach may be termed chemo-prevention and is of obvious importance, for example, in the prevention of lung cancer induced by cigarette smoking.

Alpha-fetoprotein (AFP) Studies

Quantitative serum levels of AFP have been determined by a sensitive double-antibody radioimmunoassay. The threshold level for this assay being 5 ng/ml has allowed the determination of serum levels in normal monkeys and in monkeys receiving a variety of carcinogens. The median value in *M. mulatta* was 15.5 ng/ml with a range of 12.5-22.0 and in *M. fascicularis* was 13.0 with a range of 9.0-16.5 ng/ml. Serial serum determinations for AFP were performed in monkeys receiving more than 22 different carcinogens. The majority of these agents appear not to cause hepatocellular carcinoma in monkeys and serum from these animals is evaluated on an annual basis. Serum AFP levels on these animals has remained within normal limits. In five of the carcinogen treatment groups, the likelihood of liver malignancy is greater and the AFP levels were determined at monthly or bimonthly intervals. Using the DENA treated group as an example, it was shown that a rising serum AFP preceded the clinical and histologic diagnosis of hepatoma by several months. In addition to being a useful marker for early diagnosis in these high-risk groups, AFP can also be used for following the disease course after hepatoma has developed. The serum level appears to be a valid reflection of the tumor activity and shows a dramatic fall after surgical excision of the tumor and a lesser decline following chemotherapeutic agents. Interesting correlations with traditional liver function studies were also made which showed that while alkaline phosphatase, SGOT and serum bilirubin values reached a peak 2-4 days after an initial DENA injection, the AFP response was greatest at 6-9 days and typically showed a drop for the 1st and 2nd days following DENA. This would be an indication of AFP being a response to hepatic repair as opposed to damage and destruction of hepatic cells measured by the other tests. The catabolism of AFP has been studied under several different circumstances. Twelve newborn monkeys have been followed for the first several months of life with frequent serum determinations. Levels at birth of 3,700-45,000 ng/ml fall rapidly to around 100 ng/ml by the end of one month; during the next 1-2 months the serum levels gradually come down to the normal adult range. The "half-life" of AFP during the first 30 days is between 3 and 4 days, assuming that synthesis has ceased. The half-life of AFP in a monkey with an Aflatoxin B_1 induced hepatoma following surgical removal of the tumor also was in the range of 3 days. Synthesis of AFP has been studied in fresh hepatoma biopsy specimens and in cell lines in tissue culture. Conditions to provide for linear AFP synthesis for 24-48 hours have been determined. This might therefore be a close approximation of the synthetic rate *in vivo*. Three different tumors varied between 3.3 and 7.0 ng AFP/mg tumor/day. Further studies will continue on the use of AFP as an aid to therapy of liver cancer, for chemotherapy of preneoplastic lesions and for study of its synthesis and metabolism *in vitro* and *in vivo*. Surgery is the only modality which has consistently reduced the mass of primary liver tumor and serum alpha-fetoprotein by more than 50% although several chemotherapeutic agents have reduced either the tumor mass and/or the AFP level by more than 50% in certain animals. At the present time several new antitumor drugs are being evaluated for their effects on primary liver carcinoma.

Publications:

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2. Waldman, T., McIntire, K. R., Dalgard, D. W. and Adamson, R. H.: The metabolism of tumor related proteins. In Bianchi, R., Mariani, G., and McFarlane, A. S. (Eds.): *Plasma Protein Turnover*. Baltimore, Md., University Park Press, 1976, pp. 155-164.

3. Mariani, G., Sullivan, P. W., McIntire, K. R., Adamson, R. H. and Waldman, T. A.: Purification and labelling of simian alpha-fetoprotein for tracer turnover studies. In Peeters, H. (Ed.): *Protides of the Biological Fluids*, New York, Pergamon Press, 1976, pp. 249-253.

4. Adamson, R. H., Krowlikowski, F. J., Correa, P., Sieber, S. M. and Dalgard, D. W.: Carcinogenicity of 1-methyl-1-nitrosourea (MNU) in non-human primates. *J. Natl. Cancer Inst.* (In press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 06108-08 LCHP

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)
Studies on Mechanism of Action, Mechanisms of Resistance and Anti-Metastatic Activity of Anticancer Agents

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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OTHER:	P. E. Gormley	Clinical Associate	LCP	NCI
	S. M. Sieber	Pharmacologist	LCP	NCI
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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Studies were performed to elucidate the mechanism of cytotoxicity of AMSA (NSC-141549) and to determine the basis for selective toxicity exhibited by this agent in vivo. A rational approach to achieving selective toxicity was employed for AMSA against liver metastases and hepatocellular carcinoma utilizing detoxification mechanisms present in normal, fully differentiated liver cells but not present in certain tumors. In a continuing study, several additional bis-quinaldine derivatives were synthesized and their binding to DNA, inhibition of polymerizing enzymes, in vitro cytotoxicity and in vivo antitumor activity determined. The enzymatic activation of two new antitumor agents [3-deazauridine (NSC-126849) and aminothiadiazole (NSC-4728)] was studied in normal and malignant cells. An assay was developed for quantitating the phosphorylation of 3-deazauridine in vitro and a comparison of its substrate activity with enzymes of various cells determined. In an attempt to elucidate the mechanism by which nicotinic acid reverses the toxic effects of aminothiadiazole a new rapid assay was developed for nicotinic acid phosphoribosyltransferase. However, aminothiodiazole was found not to be a substrate for this activating enzyme.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives:

The knowledge of the mechanism of action of anticancer agents and why cells become resistant to them will aid in the design of new chemotherapeutic drugs and will aid in the treatment of neoplastic diseases. Specifically:

To determine the mechanism of action of various anticancer agents.

To determine the mechanism of resistance to various anticancer agents.

To design antitumor agents that will inhibit metastases or tumor growth by affecting blood vessels and fibrin cross-linking.

To rationally design new anticancer agents.

Methods Employed:

Usual biologic, pharmacologic, and biochemical techniques. Among these are tumor transplantation, harvesting of cells, tissue culture techniques, separation and analyses of whole cell DNA, RNA, and protein synthesis. Various enzymes are isolated, purified and the effect of various antitumor agents as inhibitors of these enzymes are studied as well as effects on DNA, RNA and various proteins. Molecular models are also used in drug design.

Major Findings:

Mechanism of Action of 4'-(9-acridinylamino)-methanesulphon-m-anisidide (AMSA, NSC 141549)

The interaction of AMSA with DNA and its effects on nucleic acid polymerizing enzymes were examined in an attempt to define the site of cytotoxicity of AMSA. Binding of AMSA to DNA, as demonstrated by equilibrium dialysis and spectrophotometric methods, appears to be similar to other aminoacridines. Fluorescence studies indicated interactions between AMSA and the purine and pyrimidine rings of DNA suggestive of intercalation. DNA melting studies showed AMSA stabilized the DNA double helix but not to the same extent as other aminoacridines. Interaction of AMSA with nucleic acid polymerizing enzymes (mouse embryo DNA polymerase, AMV reverse transcriptase) was studied. Inhibition of these enzymes required AMSA concentrations of 200 to 400 μg/ml. Enzyme inhibition was greatest with templates containing only adenine-thymine base pairs. However, the relatively high concentrations of AMSA required for inhibition of these enzymes *in vitro* as compared to concentrations of AMSA necessary for cytotoxicity *in vitro* or for antitumor activity *in vivo* suggest that the interaction with DNA alone might not fully explain its marked chemotherapeutic effectiveness.

Studies on the Selective Toxicity of AMSA (NSC-141549)

Studies on the pharmacologic disposition of AMSA (see Project No. Z01 CM 03506-14 LCP) demonstrated that AMSA was selectively localized in the liver following oral administration with reduced exposure to other tissues. The in vivo detoxification of AMSA by liver involves: direct interaction with glutathione-S-transferase, non-enzymatic chemical reaction with glutathione, and active transport of metabolites into the bile. Total cellular content of glutathione-S-transferase appears to be directly related to the extent of differentiation (constitutes 5-10% of normal liver protein and absent or barely detectable in fetal liver, monkey hepatocellular carcinoma or L1210 leukemia). Thus it was anticipated that orally administered AMSA might prove to be selectively toxic against liver metastases or hepatocellular carcinoma because of impaired detoxification mechanisms in these tumors. To test this hypothesis, L1210 cells were injected iv into mice to mimic liver metastases. The mice were treated orally 5 days later, and the livers bioassayed 24 hours after treatment. A dose of 500 mg/kg (that was non-toxic and inactive against ip implanted L1210) reduced the population of L1210 cells from 2.5×10^6 cells/liver in untreated mice to undetectable levels in 6 out of 10 treated mice. Studies are presently underway to assess the oral effectiveness of AMSA in monkeys bearing chemically induced hepatocellular carcinomas. Effectiveness of treatment is being followed by X-ray scanning, α-fetoprotein levels and liver biopsy in 5 monkeys.

Synthesis and Biologic Properties of Several Bis-Quinaldine Derivatives

A series of bis-quinaldine derivatives were synthesized consisting of two substituted quinaldine moieties coupled by an alkyl bridge. These compounds possess many of the structural attributes of double or polyintercalating agents. Such a molecule can bind at two positions in the same DNA molecule or bind at two different strands of DNA. These compounds were found to be extremely toxic in vitro, to inhibit DNA-dependent RNA polymerase, to stabilize DNA as determined by Tm measurements and to exhibit in vivo anti-tumor activity against P-388 leukemia (T/C% of 110 to 186%).

Studies on 3-Deazauridine (NSC-126849)

A comparison of the metabolism of deazauridine by normal and leukemic cells in vivo revealed no qualitative differences between the different cell types. However, there was a quantitative difference in the formation of deazaUTP between normal and malignant cells. After a therapeutic dose of deazauridine in mice (300 mg/kg, ip), concentrations of deazaUTP in normal tissues ranged from 0.25 to 3.1×10^{-5}M at 30 min and 0.28 to 0.64×10^{-5}M at 240 min, after injection. In contrast, the concentrations of deazaUTP in L1210 cells was 46.8×10^{-5}M at 30 min, 30.3×10^{-5}M at 60 min, 27.6×10^{-5}M at 120 min and 19.8×10^{-5}M at 240 min. DeazaUTP inhibits CTP synthetase (thought to be the site of action of deazauridine) with a K_I of 5.3×10^{-6}M.

Thus, inhibition of CTP formation would be expected to be transient in normal tissues but prolonged in L1210 cells. Deazauridine was found to be a substrate for uridine kinase isolated from normal mouse tissues and L1210 cells, exhibiting Km's of 3.85×10^{-5}M and 8.44×10^{-5}M for uridine kinase from mouse kidney and L1210 cells, respectively. In comparison, the Km for uridine was 1.88×10^{-5}M and 4.85×10^{-5}M for uridine kinase from mouse kidney and L1210 cells, respectively. There was no correlation between tissue levels of deazauridine nucleotides and levels of uridine kinase, an enzyme that could possibly function to "trap" nucleosides intracellularly as the nucleotide form. This lack of correlation supports a recent hypothesis that cellular transport of uridine (and hence, deazauridine) plays a greater role in determining cellular concentrations of uridine nucleosides and nucleotides than does phosphorylation.

Studies on Aminothiadiazole (AT, NSC-4788)

The *in vivo* toxicity and antitumor properties of AT in mice are reversed by nicotinic acid. If AT is activated to a form where it functions as a co-enzyme analog, then activation might occur via enzyme pathways utilized for activation of nicotinic acid. A rapid enzyme assay was developed for nicotinic acid phosphoribosyltransferase utilizing DEAE discs and was compared with the previously reported time-consuming assay which utilized a chromatographic separation. Enzyme activity was measured in mouse tissues and L1210 cells. C^{14}-AT was found not to function as a substrate for these enzymes. The possible interaction of AT with other enzymes that function to convert pyridine derivatives to active co-enzyme forms is presently under investigation.

Publications:

1. Sinha, B. K., Cysyk, R. L., Millar, D. B. and Chignell, C. F.: Synthesis and Biological Properties of Some Spin-Labeled 9-Aminoacridines. *J. Med. Chem.* 19: 994-998, 1976.

2. Shoemaker, D. D., Legha, S. S. and Cysyk, R. L.: Selective Localization of 4'-(9-Acridinylamino)-methanesulfon-m-anisidide in B16 Melanoma. *Pharmacology.* (in press).

3. Gormley, P. E., Benvenuto, J. and Cysyk, R. L.: Synthesis and Biologic Studies of Arabinosylcytosine 5'-Methylphosphonate. *Biochemical Pharmacology.* (in press).

4. Sinha, B. K., Philen, R. P., Sato, R. and Cysyk, R. L.: Synthesis and Antitumor Properties of Bis-Quinaldine Derivatives. *J. Med. Chem.* (in press).

5. Gormley, P. E., Sethi, V. S. and Cysyk, R. L.: Interaction of 4'-(9-acridinylamino)methanesulphon-m-anisidide (AMSA) with DNA and Inhibition of Oncornavirus Reverse Transcriptase and Cellular Nucleic Acid Polymerases. Cancer Research. (in press).

6. Cysyk, R. L. and Adamson, R. H.: Protein Cross-Linking Properties of the Antitumor Agent Imosine Dialdehyde (NSC-118994). Cancer Treatment Rep. 60: 563-570, 1976.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 06134-02 LCHP

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

The Role of the Lymphatic System in the Absorption and Distribution of Antitumor Agents.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	S. M. Sieber-Fabro	Pharmacologist	LCP	NCI
OTHER:	R. H. Adamson	Chief, Lab.of Chem.Pharm.	LCP	NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Chemical Pharmacology

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Pharmacology and Experimental Therapeutics Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	0.5	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The role of the lymphatic system in the absorption and distribution of a variety of radiolabelled antitumor agents (CCNU, methyl-CCNU, guanazole, dibromomannitol, diethylstilbestrol, prednisolone, dexamethasone, 6-mercaptopurine, cytosine arabinoside, vincristine, cyclophosphamide, adriamycin, daunomycin, hexamethylmelamine, procarbazine, actinomycin D and o,p-DDD) in rats is under investigation. The relationship between extent of lymphatic absorption and the molecular weight and lipid solubility of the agents, their route of administration, and the vehicle or formulation in which they are given, is also being studied. Efforts are underway to develop a reproducible model system in rats for tumor metastasis via lymphatic channels, thus making it possible to evaluate the therapeutic advantage of treating such tumors with antitumor agents selectively taken up by lymphatics.

Project Description:

Objectives:

This project was initiated in order to provide information on the role of the lymphatic system in the absorption and distribution of antitumor agents, and to develop a model system in rats in which to study tumor metastasis via lymphatic channels. Specifically, the objectives are:

To ascertain the extent to which a wide variety of clinically useful antitumor agents are carried by lymphatic channels.

To determine whether there are specific physico-chemical characteristics which a compound must possess to ensure its preferential absorption and/or distribution by lymphatic vessels. In addition, studies are underway to examine whether the lymphatic uptake of a compound can be influenced by the route of its administration and by the vehicle or formulation in which it is given. Especial emphasis will be given to studies on the lymphatic distribution of selected lipid-soluble antitumor agents encapsulated in small unilamellar liposomes. The information derived from these studies will guide our future efforts to design agents which are not only active against experimental tumors but are also selectively carried by lymphatics.

To develop a model system in rats in which metastatic tumor spread via lymphatic channels can be studied. Although a number of clinically important human neoplasms (e.g., ovarian and mammary carcinoma) metastasize via lymphatic channels, most transplantable rodent tumors spread by hematogenous metastasis or by local invasion. A model system in rats for tumor metastasis via lymphatic channels would make it possible to evaluate the therapeutic advantage of treating such tumors with antitumor agents selectively taken up by lymphatics.

Major Findings:

The intestinal absorption of most of the clinically-effective antitumor agents tested to date takes place primarily via the portal route rather than by the intestinal lymphatic system. Following the intraduodenal administration of radioactive CCNU, methyl-CCNU, guanazole, dibromomannitol, diethylstilbestrol, prednisolone, dexamethasone, 6-mercaptopurine, cytosine arabinoside, and cyclophosphamide, 5% or less of the administered radioactivity was recovered in 24-hour thoracic duct lymph. The only antitumor agent tested so far which appears to be selectively absorbed by the intestinal lymphatics is o,p-DDD. Following the administration of this compound in aqueous solution, 12.2% of the administered radioactivity was recovered in thoracic duct lymph, and this figure was increased to 26.1% when the compound was administered as a suspension in corn oil. An increase in lymphatic absorption of other compounds (prednisolone, dexamethasone, and 6-mercaptopurine) was also noted when they were administered as emulsions in corn oil rather than in aqueous solution, but this increase was not statistically significant. Other small molecular weight compounds of relatively high lipid solubility (CCNU, methyl-CCNU, diethylstilbestrol) were absorbed to approximately the same extent as were the relatively polar compounds (guanazole, cytosine arabinoside), regardless of whether they were given in aqueous solution or oil suspension. Compounds of relatively high molecular weight (vincristine, actinomycin D) were not more extensively taken up by lymphatic vessels than the lower molecular weight compounds. Approximately 0.4% of the administered dose of radioactivity was recovered in thoracic duct lymph of rats treated by either the intravenous or intramuscular routes with ^{3}H-vincristine. In contrast, 1.6% of the administered radioactivity was recovered in lymph from rats given ^{3}H-actinomycin D by the intramuscular route, whereas this value was increased 4-fold when the drug was given intravenously. Approximately equal amounts of cyclophosphamide equivalents (1.4-1.8% of the administered dose) were recovered in 24-hour thoracic duct lymph following the administration of ^{14}C-cyclophosphamide by intraduodenal or intravenous routes, suggesting that, at least in the case of cyclophosphamide, the lymph radioactivity derives from the distribution of cyclophosphamide equivalents in the body water, of which lymph is a part.

Publications:

1. Sieber, S. M. and Adamson, R. H.: Selection of carriers for alkylating agents to increase their antitumor specificity. Cancer Treatment Rep. 60: 217-219, 1976.

2. Sieber, S. M.: The lymphatic absorption of p,p'-DDT and some structurally-related compounds in the rat. Pharmacology. 14: 443-454, 1976.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 06136-01 LCHP

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Drug induced cell differentiation in neoplasia.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	S. S. Thorgeirsson	Head, Biochemical Pharma. Sect.	LCP	NCI
OTHER:	P. J. Wirth	Staff Fellow	LCP	NCI
	H. A. J. Schut	Visiting Fellow	LCP	NCI
	S. Sakai	Visiting Fellow	LCP	NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Chemical Pharmacology

SECTION
Biochemical Pharmacology Section

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NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.5	2.0	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This study was initiated to understand the mechanism of re-differentiation of neoplastic cells by drugs and other chemical agents. The research is directed at present to determine: (1) the effect of dimethylsulfoxide (DMSO) and its analogs on the expression of Glutathione synthesis in hepatoma cell lines in culture and also transplantable hepatomas; (2) the effect of polar compounds such as DMSO, bisacetamides and antitumor agents on fetal expressions (such as α-feto-protein) in hepatomas and CEA in colon tumors; (3) the reversibility, when exposed to the above mentioned agents, of choriocarcinoma in tissue culture using HCG as marker; and (4) the role of the compounds mentioned above in redifferentiation of neuroblastoma cell lines.

Project Description:

Objectives:

The objective of this project is to examine the feasibility of using chemically induced cell differentiation as a therapeutic approach to neoplastic diseases either alone or in combination with established chemotherapeutic agents. This new approach is aimed at finding chemicals with the capacity to "redifferentiate" or normalize transformed malignant cells. Information already available indicates that this approach is feasible. For example, the addition of DMSO to cultures of murine-virus induced erythroleukemia cells results in erythroid differentiation characterized by the appearance of globin mRNA, synthesis of hemoglobin, cessation of cell division, appearance of erythrocyte membrane antigens, and the morphological changes characteristic of erythroid differentiation in normal mouse hemopoietic tissue.

Methods Employed:

Methods used in these studies include: tissue culture technique, radioisotope measurements using tritium and carbon-14; phase microscopy; enzyme assays involving radiometric spectrophotometric and spectrophotofluorometric determinations; polyacrylamide gel electrophoresis; and chemical synthesis.

Major Findings:

So far two major findings have emerged: (1) Glutathione synthesis is 4-6 times slower in tumor cells than in the corresponding normal cells and (2) DMSO treatment of tumor cells in tissue culture increases the rate of glutathione synthesis.

Significance to Biomedical Research and the Program of the Institute:

Our studies indicate that polar compounds such as DMSO can induce the neoplastic cell to "redifferentiate" and perhaps loose its malignant characteristics. Furthermore, the observations that glutathione synthesis is much slower in the neoplastic cell, may provide a way to increase the selective toxicity of some therapeutically used alkylating agents. For example, glutathione (GSH) can be depleted in both normal and neoplastic tissues by a variety of agents. Since the synthesis of GSH is much slower in the neoplastic tissue, the concentration of GSH in the neoplastic tissue will be much lower than normal at the time point when GSH concentration in healthy tissues is normal or even higher than normal. These findings could increase, when applied in cancer chemotherapy, both the safety and the effectiveness of the treatment.

Proposed Course:

To continue research on the effect of polar chemical compounds, antitumor agents and steroids on cell differentiation in a variety of tumor systems.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 06137-01 LCHP

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Mechanism of Chemically Induced Hepatomas and Ovarian Tumors

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	S. S. Thorgeirsson	Head, Biochemical Pharm. Sect.	LCP	NCI
OTHER:	D. R. Mattison	Research Associate	LCP	NCI
	R. W. Downs	Clinical Associate	MD	NIAMD

COOPERATING UNITS (if any)

Metabolic Disease Branch, NIAMD

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INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS: 2.5	PROFESSIONAL: 2.0	OTHER: 0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The long range purpose of this project is to study the mechanism of chemically induced hepatomas and ovarian tumors in rodents and non-human primates. We are particularly interested in the role of environmental contaminants such as the polycyclic hydrocarbons, nitroso-compounds and N-acetylarylamines in initiating these tumors, as well as the role of genetic predisposition in development of these tumors. These tumors, at various time points during their development, will then be used as models for evaluating chemotherapeutic agents and other drugs which might be of therapeutic value. Topics of present interest are: (1) The role of metabolic activation, as well as the genetic control of activating enzymes, in the chemical initiation of hepatoma and ovarian tumors; (2) The time course of hepatoma and ovarian tumor initiation with particular emphasis on the changing enzyme patterns as the cells become increasingly dedifferentiated; (3) The role of nucleophilic agents such as N-acetylcysteine, L-cysteine and cysteamine in preventing the chemical initiation of hepatomas and ovarian tumors; (4) The characterization of cAMP and the β-receptor involvement in the chemical induction of hepatomas as well as the possible use of β-blockers and/or β-agonists in preventing the occurance of these hepatomas.

Project Description:

Objectives:

The main objectives of this project are: (1) to study in rodents and non-human primates the relationship between the activity, both basal and induced, of the enzymes which metabolically activate compounds such as polycyclic hydrocarbons, N-acetylarylamines, and nitroso-compounds, and toxicity as well as carcinogenicity of these compounds. The main emphasis will be focused on the liver, the ovary and the GI tract; (2) to examine the role of enzyme induction of aryl hydrocarbon hydroxylase and N-acetylarylamine N-hydroxylase, which is known to be inherited as an autosomal dominant trait in mice, in toxicity and carcinogenicity of polycyclic hydrocarbon and N-acetylarylamines. The importance of these studies with respect to man is highlighted by reports indicating that inducibility of aryl hydrocarbon hydroxylase in cultured peripheral lymphocytes is correlated with the occurance of bronchogenic carcinoma. Furthermore, the 3-4 fold increase in ovarian cancer over the last 40 years in the U.S.A., with the highest incidence in urban industrialized areas, indicate that part of this increase may be caused by environmental contaminants; (3) to clarify and define the role of nucleophilic agents such as N-acetylcysteine, L-cysteine and cysteamine in protecting against toxicity and carcinogenicity of antitumor agents and chemical carcinogens; (4) to study the time course of hepatoma and ovarian cancer initiation. The main emphasis will be on the changing enzyme patterns as the cells become increasingly de-differentiated, and the effect of established antitumor agents as well as other compounds of therapeutic interest on the development of these tumors; (5) to examine the role of cAMP and the β-receptor in the chemical initiation of hepatomas. The possibility that β-blockers orβ-agonists might be of therapeutic value in preventing initiation of hepatomas is also under study.

Methods Employed:

The principal methods employed are: (1) recording spectrophotometry of turbid solutions, (2) enzyme assays involving radiometric, spectrophotometric, and spectrophotofluorometric determinations of product formation or substrate disappearance, (3) radioisotopic measurements using tritium and carbon-14, (4) differential centrifugation, (5) polyacrylamide gel electrophoresis, (6) column and thin-layer chromatography, and (7) high pressure liquid chromatography.

Major Findings:

1. Induction of content and activity of hepatic and lung cytochrome P-450 dependent monooxygenases by 3-methylcholanthrene has been studied in male rhesus monkeys. Hepatic microsomes were concomitantly studied for mutagenic activiation of 2-acetylaminofluorene as well as with the use of sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Hepatic N-hydroxylation

of 2-acetylaminofluorene is increased 6-7 fold following 3-methylcholanthrene treatment; similar increase is observed for aryl hydrocarbon hydroxylase in both liver and lung. The hepatic cytochrome P-450 content is increased by 50% and the Soret maximum in the CO-hemoprotein complex is shifted to the blue by 2 nm after 3-methylcholanthrene induction.

Electrophoresis of hepatic microsomes from 3-methylcholanthrene treated monkeys shows increase in a polypeptide band of 54,000 molecular weight. Mutagenic activation of 2-acetylaminofluorene by liver microsomes from untreated rhesus monkeys is very low but is increased 40 fold after 3-methylcholanthrene treatment. No effect is observed on the mutagenic activation of N-hydroxy-2-acetylaminofluorene by liver microsomes following 3-methylcholanthrene treatment.

These studies may explain the lack of carcinogenic activity of 2-acetylaminofluorene in rhesus monkeys.

2. The ovary from rodents such as the mouse and the rat can metabolize polycyclic hydrocarbons such as benzo[a]pyrene, methylcholanthrene and dimethylbenzanthracene. The enzyme(s) system has been characterized as cytochrome P-450 dependent monooxygenase, which is inducible by polycyclic hydrocarbons. The responsiveness of the mouse ovarian monooxygenases (aryl hydrocarbon hydroxylase) to polycyclic hydrocarbon treatment is the same as has been shown for liver, lung and other organs, i.e. the ability to induce is inherited as an autosomal dominant trait. Another interesting finding relates to the fact that the degree of polycyclic hydrocarbon destruction of primordial oocytes is closely correlated with the activity of the ovarian aryl hydrocarbon hydroxylase. This is of importance, since the first step in the initiation of granulosa cell tumors in mice is the destruction of primordial oocytes.

3. Preliminary experiments indicate that cysteamine, and to a lesser degree L-cysteine and N-acetylcysteine, protects against melphalan toxicity (using death as an end-point).

4. Catecholamine stimulation of adenyl cyclase (200-300% increase) in rat liver is observed during the first few weeks when the animals are fed hepatocarcinogen such as 2-acetylaminofluorene. The catecholamine stimulation of cAMP is normally observed only during fetal life and the early neonatal period. We also find increase in the β-receptor concentration during the same period. This is an exciting finding since it opens the possibility that β-blockers or β-agonists may have a place in cancer chemotherapy.

Significance to Biomedical Research and the Program of the Institute:

Our studies are aimed at providing better understanding of tumor initiation as well as tumor progression. This information will then be used to design chemotherapeutic regimens as well as new chemotherapeutic agents for treating these and other tumors.

Proposed Course of Project:

Continue the course outlined under objectives.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 06138-01 LCHP

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Mutagenicity and Carcinogenicity of Antitumor Agents and Chemical Carcinogens

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	S. S. Thorgeirsson	Head, Biochemical Pharma. Sect.	LCP	NCI
OTHER:	S. Sakai	Visiting Fellow	LCP	NCI
	H. A. J. Schut	Visiting Fellow	LCP	NCI
	E. Dybing	National Institute of Public Health, Oslo 1, Norway		

COOPERATING UNITS (if any)

National Institute of Public Health, Oslo 1, Norway

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Laboratory of Chemical Pharmacology

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Biochemical Pharmacology Section

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NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS: 2.0	PROFESSIONAL: 2.0	OTHER: None

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project was initiated to evaluate the relationship between chemically induced mutagenesis and carcinogenesis. The primary emphasis is placed on antitumor agents and known environmental contaminants and model chemical carcinogens. A sensitive in vitro bacterial test system is used for the detection of chemical mutagens. The research is directed at present to determine: (1) the role, if any, of metabolic activation in the mutagenesis caused by antitumor agents and chemical carcinogens; (2) the effect of structural modification on the mutagenicity of both antitumor agents and model chemical carcinogens; (3) the feasibility of using nucleophiles such as L-cysteine, N-acetyl-cysteine and cysteamine to inhibit mutagenicity of the above mentioned compounds.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives:

Recently, Ames and coworkers (Berkeley University) have developed a sensitive bacterial test system for detection of chemical mutagens in vitro. This system has been used as a screening test for chemical carcinogens, and over 90% of the known carcinogens were shown to be mutagens in this system. We have used this system for detecting mutagens in our studies. The main objectives of this project are: (1) to study the relationship between mutagenicity and carcinogenicity of antitumor agents. Of particular interest to us are those agents (such as melphalan and procarbazine) which are known to cause tumors in both experimental animals and in humans following chemotherapy; (2) to examine the role of both microsomes and nuclear membrane from hepatocytes in activating procarcinogens to ultimate carcinogens and/or mutagens capable of binding to cellular macromolecules; (3) to study the feasibility of using nucleophilic agents such as glutathione, L-cysteine, N-acetylcysteine and cysteamine to inhibit and/or modify the mutagenic potential of antitumor agents.

Methods Employed:

The principal methods employed are: (1) bacterial culture technique, (2) differential centrifugation, (3) enzyme assays, (4) recording spectrophotometry, and (5) high pressure liquid chromatography.

Major Findings:

1. The alkylating agents so far tested in the bacterial test system have proven to be very toxic to the bacteria. This has led to some technical difficulties which we have now overcome.

2. Esterases, both in the microsomes and in the nuclear membrane from liver, kidney and intestinal mucosa, are very effective in activating chemical carcinogens such as N-hydroxy-2-acetylaminofluorene to a very potent mutagen(s).

3. The activation of the hair dye 2,4-diaminoanisole to a mutagen in the bacterial test system by liver fractions of rats and mice is increased by treatments of animals with inducers of the cytochrome P-450 system and is decreased in in vivo and in vitro inhibitors of cytochrome P-450. Higher revertant rates were seen with 2,4-diaminoanisole in aromatic hydrocarbon responsive mice than in nonresponsive mice after treatment with β-naphthoflavone. Mutagenic activity of 2,4-diaminoanisole is also induced in kidney and lung as well as transplacentally in fetal liver after treatment with β-naphthoflavone. We suggest that metabolic activation of 2,4-diaminoanisole to the hydroxylamine(s) may be the underlying reaction for the formation of mutagenic intermediates.

4. Hydrazine compounds, including procarbazine, have been found to have low mutagenic potential when activating enzymes from mouse are used. Further studies with enzymes from rats and monkeys are underway.

Significance to Biomedical Research and the Program of the Institute:

Our studies are aimed at providing better understanding of the mechanism of toxicity and potential carcinogenicity caused by antitumor agents and environmental pollutants. This information will provide a sounder base for designing chemotherapeutic regimes, and could provide means to prevent toxic side-effect of these agents.

Proposed Course of Project:

Continue the course outlined under objectives.

Publications:

1. Dybing, E. and Thorgeirsson, S. S.: Metabolic Activation of 2,4-Diaminoanisole, a Hair-Dye Component - I. Role of Cytochrome P-450 Metabolism in Mutagenicity *In Vitro*. *Biochemical Pharmacology*. 26: 729-734, 1977.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 06139-01 LCHP

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Studies on the Therapeutic Use of Adenosine Deaminase Inhibitors

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	R. H. Adamson	Chief, Lab. of Chem. Pharma.	LCP	NCI
OTHER:	D. G. Johns	Chief, Lab. Med. Chem. & Biology	LMCB	NCI
	M. M. Chassin	Clinical Associate	LMCB	NCI

COOPERATING UNITS (if any)

Laboratory of Medicinal Chemistry and Biology, NCI

LAB/BRANCH
Laboratory of Chemical Pharmacology

SECTION
Office of the Chief

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS: 1.0	PROFESSIONAL: 0.5	OTHER: 0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES XX(c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The potent adenosine deaminase inhibitor, 2'-deoxycoformycin, inhibits the enzymic inactivation and potentiates the cytotoxic activity of a variety of adenosine analogs in the P388 murine leukemia cell culture system. Tubercidin (7-deaza-adenosine) is not a substrate for adenosine deaminase and its cytotoxicity in vitro was not potentiated by 2'-deoxycoformycin. Both 2'-deoxycoformycin and erythro-9-(2-hydroxy-3-nonyl)-adenine (EHNA) potentiate the antitumor activity of xylosyladenine against leukemia P388 and produce long term survivors. In addition 2'-deoxycoformycin was shown to have immunosuppressive activity in mice using an allograft tumor test system.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives:

This project is designed to answer two questions: (1) if inhibition of adenosine deaminase will potentiate the action of purine nucleoside antimetabolites and (2) if adenosine deaminase inhibition will also result in suppression of certain immunological functions.

Methods Employed:

Usual pharmacological, biochemical, immunological techniques and the use of animal tumors and tissue culture techniques.

Major Findings:

The compound 2'-deoxycoformycin (2'-DCF) inhibits the deamination of adenosine, 8-aza-adenosine, 6-chloropurine ribonucleoside, 3'-deoxyadenosine, formycin A, 4'-thioadenosine and 9-β-D-xylofuranosyladenine by adenosine deaminase. Tubercidin (7-deaza-adenosine) was neither a substrate nor an inhibitor for the enzyme. The cytotoxic effects of adenosine and all the adenosine analogs except 7-deaza-adenosine were enhanced by 2'-deoxycoformycin.

The antitumor activity of 3'-deoxyadenosine and of 9-β-D-xylofuranosyladenine against leukemia P388 was enhanced by 2'-DCF yielding an increase in median survival time over that compared to the adenosine analog when used alone as well as long term survivors. In addition, erythro-9-(2-hydroxy-3-nonyl)-adenine potentiates the antitumor activity of 9-β-D-xylofuranosyladenine against leukemia P388 and produces long term survivors.

Recent studies were carried out to evaluate the immunosuppressive activity of 2'-DCF, using an allograft tumor test system in which LSTRA cells (a Moloney virus-induced leukemia in BALB/c mice) were transplanted subcutaneously into C57bl/6 mice across an H-2 histocompatibility barrier. Animals were divided into four treatment groups: (1) no treatment; (2) BCNU 40 mg/kg ip 24 hours pretransplant; (3) 2'-DCF 15 mg/kg 24 hours pretransplant; and (4) 2'-DCF 0.25 mg/kg qd beginning 24 hours pretransplant. The end point was failure to reject the allograft and consequent death from progressive metastatic tumor. Using an inoculum of 2×10^6 cells all animals developed tumors. Only 1 of 15 untreated animals died of tumor; the remainder completely rejected their grafts. The positive control BCNU, known to be an effective immunosuppressant in this system, caused 12 of 15 (80%) animals to die with progressive tumor. Eleven of 16 (69%) animals in group (3) died of progressive tumor as did seven of 16 (44%) in group (4). All treated groups are significantly ($p < 0.05$) different from controls and 2'-DCF pretreatment is not significantly different from BCNU pretreatment (Fisher exact-probability test). These studies support the concept that certain immunologic functions can be suppressed in vivo by adenosine deaminase inhibitors, just as they can be restored by providing the enzyme to those deficient in it.

Significance to Biomedical Research and the Program of the Institute:

These studies were designed in order to select an adenosine analog to be evaluated in phase I clinical trial in combination with one of the adenosine deaminase inhibitors. In addition if immunosuppressive activity without significant host toxicity can be provided by administration of the adenosine deaminase inhibitors then they would be of value for organ transplants.

Proposed Course of Project:

To continue the course outlined under the objectives and to assist in the design of clinical trials of adenosine analogs in combination with an adenosine deaminase inhibitor for chemotherapy of tumors and to ascertain the importance of adenosine deaminase inhibition for organ transplants.

Publications:

1. Johns, D. G. and Adamson, R. H.: Enhancement of the Biological Activity of Cordycepin (3'-Deoxyadenosine) by the Adenosine Deaminase Inhibitor 2'-Deoxycoformycin. Biochemical Pharmacology. 25: 1441-1444, 1976.

2. Adamson, R. H., Zaharevitz, D. W. and Johns, D. G.: Enhancement of the Biological Activity of Adenosine Analogs by the Adenosine Deaminase Inhibitor 2'-Deoxycoformycin. Pharmacology. 15: 84-89, 1977.

3. Chassin, M. M., Chirigos, M. A., Johns, D. G. and Adamson, R. H.: Adenosine Deaminase Inhibition for Immunosuppression. New England J. of Medicine. 296: 1232, 1977.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 07100-04 LCHP

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Tumor Biology and Chemotherapy

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	R. K. Johnson	Head, Experi. Chemotherapy Sect.	LCP	NCI
OTHER:	M. P. Chitnis	Visiting Fellow	LCP	NCI
	M. Inaba	Visiting Fellow	LCP	NCI
	R. Osieka	Guest Worker	LCP	NCI
	R. L. Magin	IPA	LCP	NCI

COOPERATING UNITS (if any)
G. R. Stark, Stanford Univ.; K. Gerzon, Lilly Research Labs.; H. Meienhofer, Hoffman-LaRoche, Inc.; I. Wodinsky, Arthur D. Little Co.; T.S.A. Samy, Sidney Farber Cancer Center; E. Katz, Georgetown Univ.

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SECTION
Experimental Chemotherapy Section

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NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
8.0	5.0	3.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Work within this project is directed at a) determining structure-activity relationships and uncovering more effective analogues within important classes of antineoplastic agents including anthracycline and actinomycin antibiotics, trichothecene mycotoxins and Vinca alkaloids, b) detailed evaluation of new potential antineoplastic agents including PALA and inhibitors of protein synthesis, c) investigation of resistance and cross-resistance among antineoplastic agents and d) development of new bioassay systems such as human tumor xenografts and new treatment modalities such as immunotherapy and hyperthermia.

Project Description:

Objectives:

To undertake fundamental studies in therapy of experimental tumors; to investigate potential and established clinical cancer chemotherapeutic agents with respect to their effect on malignant growth and selectivity; to determine the relationship between various biological properties of tumors and response to chemotherapy; to study structure-activity relationships among major classes of antineoplastic agents; to develop underlying principles of successful cancer therapy. Specific objectives include: study of drug sensitivity differences and control mechanisms in the cell cycle; study of the origin and basis of invasiveness, metastasis and tumor angiogenesis and the effects of drugs on these phenomena; investigation of means for effective therapy and prevention of emergence of resistant cell populations; study of the mechanism of drug action and mechanism of drug-induced cell death; study of the influence of drugs on hematopoietic and other sensitive host tissues in relation to antitumor effect; development of new and improved biological systems for screening and detailed evaluation of potential antineoplastic agents; determination of underlying principles of effective combination chemotherapy and combined modality treatment.

Methods Employed:

Standard laboratory procedures were employed.

Major Findings:

I. Structure-Activity Relationships and Analogue Evaluation

A. Actinomycins

After three years of comparative evaluation in a number of tumor systems a total of 35 actinomycin analogues have been tested. None of these analogues had clear therapeutic superiority over the parent antibiotic, actinomycin D, although marginally better antitumor activity of actinomycin Pip$_{1\beta}$ was demonstrated and repeatedly confirmed in B16 melanoma. Considerable information on the structure-activity relationships among actinomycins was obtained which will be useful in further efforts to develop a superior actinomycin analog.

B. Anthracyclines

A variety of synthetic and naturally occurring anthracyclines were tested in experimental tumor systems in comparison with adriamycin. Antitumor activity was confirmed for a number of these compounds although none were clearly superior to adriamycin. Twenty-five anthracyclines have been tested for cross-resistance in a subline of P388 leukemia resistant to adriamycin. The resistant subline proved to be cross-resistant to all of the analogues.

C. Trichothecene mycotoxins

The high degree of activity of anguidine in P388 leukemia and particularly in colon carcinoma 38 has aroused a good deal of clinical interest in this mycotoxin. A closely related trichothecene, T2 toxin, and several macrocyclic analogues of this group of compounds were compared in experimental tumor systems. T2 toxin was reproducibly superior to anguidine in P388 leukemia as were three macrocyclic derivatives from *Baccharus megapotamica*, namely baccharin, B_1 and B_2. In addition the latter two compounds were quite active against B16 melanoma which is refractory to anguidine and other trichothecenes.

D. Vinca alkaloids

A number of semisynthetic analogues of vinblastine were obtained from the Lilly Research Laboratories and compared with vincristine, vinblastine and vindesine in experimental tumor systems. A subline of P388 leukemia resistant to vincristine retained sensitivity to N-β-mercaptoethylvindesine yet was cross-resistant to vindesine and the closely related N-β-hydroxyethylvindesine.

II. Detailed Evaluation of New Potential Antitumor Agents

A. N-(Phosphonacetyl)-L-aspartic acid (PALA)

This rationally synthesized inhibitor of aspartate transcarbamylase was tested in a spectrum of experimental tumors. It proved to be a solid tumor-specific antimetabolite with a high degree of activity against six of the eight solid tumors tested (Lewis lung carcinoma, B16 melanoma, Glioma 26, M5076 ovarian carcinoma, C3H mammary carcinoma, colon carcinoma 26) but was inactive in four leukemias. The basis for this selective effect on solid tumors was shown to be related to the levels of the target enzyme which are lower in the solid tumors. Reversal of toxic and antineoplastic effects *in vivo* was demonstrated with administration of carbamyl aspartate thus proving that the effects of this drug are due to inhibition of aspartate transcarbamylase.

B. *Corynebacterium parvum*

This immunostimulant was shown to be active when used alone against P388 leukemia and B16 melanoma, tumors which do not respond to other immunostimulants such as BCG and levamisole.

C. Inhibitors of protein synthesis

A number of natural products have been developed for clinical trial and were later shown to be protein synthesis inhibitors. In the past this group of drugs has failed to show activity at tolerated doses in Phase I and II trials (i.e. emetine, puromycin, pactamycin). Four drugs currently under development for or in clinical trial are protein synthesis inhibitors (anguidine, bruceantin, homoharringtonine and bouvardin). The latter agent was shown to be a protein synthesis inhibitor in studies in our laboratory. To better understand the antineoplastic activity of this class of drugs we have studied their cytotoxicity as related to extent and duration of protein synthesis inhibition. With a number of structurally-unrelated compounds it was found that protein synthesis had to be completely inhibited for more than 4 hours to give more than 3 log cell kill. The studies indicate the importance of maintaining effective plasma levels after administration of these agents to tumor-bearing animals.

III. Resistance and Cross-Resistance to Antitumor Agents

A. Characteristics of sublines of P388 leukemia resistant to adriamycin and daunorubicin

The cross-resistance pattern and mechanism of resistance of P388/ADR and P388/DAU was further characterized. Resistance to adriamycin, daunorubicin, actinomycin D and emetine was shown to be due to decreased retention of these drugs within the resistant cells. Using actinomycin D it was shown that binding of the drug to isolated nuclei or isolated chromatin was identical in sensitive and resistant cells. The basis for decreased retention appears to be associated with an efflux mechanism involving the plasma membrane.

B. Characteristics of a subline of P388 leukemia resistant to vincristine

P388/VCR was evaluated for cross-resistance to a spectrum of drugs previously evaluated against P388/ADR. P388/VCR was shown to be sensitive to some Vinca alkaloid derivatives and to other mitotic spindle poisons such as the epipodophyllotoxins. Although it was cross-resistant to all protein synthesis inhibitors (similar to P388/ADR) there were a number of agents active against P388/VCR that were cross-resistant in P388/ADR (i.e. ICRF-159, hycanthone, ellipticine) and vice-versa (i.e. anthramycin, indicine N-oxide). These findings suggest that the mechanism of resistance in these two cell lines is dissimilar.

IV. Chemotherapy of Human Colon Carcinoma Xenografts in Athymic Nude Mice

An effort was made to test new drugs under development for clinical trial against a panel of three colon carcinoma xenografts. These tumors failed to respond to a number of agents with a variety of mechanisms and exciting activity against murine tumors. The results indicate that colon carcinoma xenografts are valid chemotherapy models for the human disease based on poor response to chemotherapy.

V. Development of Local Microwave Hyperthermia as a Treatment Modality

A program was initiated to develop a 2450 MHz microwave apparatus for the controlled local application of hyperthermia to murine tumors. The project was directed at perfecting applicators and servo mechanisms for heating solid tumors up to 1 gm in mass. Preliminary studies with murine mammary carcinomas and colon carcinoma xenografts showed that an oncolytic effect could be achieved but temperature nonuniformity resulted in variability of response. Current efforts are geared toward development of a system which will uniformly heat peripheral and visceral tumors.

Significance to Biomedical Research and the Program of the Institute

Work on this project is directed primarily toward development of new drugs and other modalities for the treatment of cancer and secondarily toward gaining a better understanding of the principles of effective chemotherapy. The new antimetabolite, PALA, is being developed for clinical trial based on investigations within this project. The evaluation of chemotherapeutic agents against human xenografts in this project has formed a basis for large-scale screening of drugs in this system. Studies of resistance in P388/ADR have uncovered a new mechanism of resistance to important classes of antineoplastic agents.

Proposed Course of Project

During the next year efforts will continue in each of the areas described above. Increased emphasis will be placed on determining the basis for decreased retention of drugs in the anthracycline-resistant tumor cells, evaluating the effectiveness of microwave hyperthermia as a treatment modality in a spectrum of animal tumor systems, biochemical pharmacology of PALA, and detailed evaluation of cytotoxic protein synthesis inhibitors with emphasis on the trichothecene mycotoxins. Drug evaluation in human xenografts will be extended as much as possible as limited by the supply of athymic nude mice.

Publications:

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8. Osieka, R., Houchens, D. P., Goldin, A. and Johnson, R. K.: Chemotherapy of human colon cancer xenografts in athymic nude mice. *Cancer*. (In press).

9. Samy, T. S. A., Hu, J-M., Meienhofer, J., Lazarus, H., and Johnson, R. K.: A facile method of purification of neocarzinostatin, an antitumor protein. *J. Natl. Cancer Inst*. (In press).

10. Wodinsky, I., Swiniarski, J. K., Venditti, J. M. and Johnson, R. K.: The effectiveness of sequential therapy schedules with adriamycin and cyclophosphamide in the P388 leukemia model. In Hellmann, K. and Connors, T. A. (Eds.): *Chemotherapy, Proceedings IX International Congress Chemotherapy*, vol. 8. New York, Plenum Press, 1976, pp. 63-76.

Appendix A

Experimental Chemotherapy Section
Contracts

Contract No. N01-CM-57030

Principal Investigator: Dr. Arthur E. Bogden

Co-principal Investigator: Dr. Diane Kelton

Mason Research Institute

$166,471

Objectives:

To develop new in vivo tumor systems with potential predictive value in selecting drugs which will be active in human solid tumors, to evaluate growth characteristics, cytokinetic parameters and chemotherapeutic response to potential and established antitumor drugs of transplantable animal tumors with special emphasis on human tumor xenografts in nude mice.

Major Findings:

An assay for quick evaluation of agents against human tumor xenografts was developed which would be adaptable to large-scale screening. This technique involved implantation of tumors under the renal capsule to evaluate drug effects on minimal tumor burden and over a short time period. The technique proved to be reproducible and to segregate highly active from inactive drugs. Several rodent tumor systems including ovarian, bladder, testicular, lung (adenocarcinoma) and prostatic tumors were characterized. The ovarian tumor was shown to be highly responsive to hexamethylmelamine and some other clinically useful agents which have poor activity in murine tumor systems in general.

Significance to Biomedical Research and the Program of the Institute:

Experimental tumor systems currently in use do not select, with any degree of precision, agents with activity against common human solid tumors. It would be beneficial to have a spectrum of murine tumors which more closely resemble human carcinomas in biology and chemotherapeutic response. This contract is designed to develop such tumor systems.

Proposed Course of Project:

This contract will continue to explore both human xenografts and murine solid tumors as drug evaluation systems. As the colony of nude mice expands there will be greater emphasis on chemotherapy of xenografts and less emphasis on murine tumors.

Contract No. N01-CM-53767

Principal Investigator: Dr. Roger N. Rosenberg

Co-principal Investigator: Dr. Fred Baskin

University of Texas Health Science Center at Dallas

$76,773

Objectives:

To develop tumor cell sublines resistant to drugs of interest to the National Cancer Institute, determine patterns of cross-resistance among established and potential antineoplastic agents, and evaluate mechanism of resistance to selected agents.

Major Findings:

Sublines of mouse C1300 neuroblastoma resistant to the following drugs were obtained and characterized in tissue culture: AT-125 (NSC 163501), L-alanosine, Baker's antifol, maytansine, vincristine, anguidine and adriamycin. Studies with labelled vincristine and colchicine suggested that the maytansine-resistant line was resistant by virtue of decreased uptake. The anguidine-resistant line was tested for cross-resistance to a number of related trichothecene mycotoxins. One of the agents, baccharin, proved not to be cross-resistant. The Baker's antifol-resistant line was not cross-resistant to methotrexate but was cross-resistant to adriamycin and vincristine. This line did not have elevated DHFR levels.

Significance to Biomedical Research and Program of the Institute:

This contract, in supplying sublines with defined mechanisms of resistance and patterns of cross-resistance, will provide tools for determining whether chemical analogues of established drugs or unique structures are, in fact, biochemical analogues. In addition this contract will supply basic information on the cytotoxic activity and mechanism of action of unique antineoplastic agents.

Proposed Course of Project:

Efforts will continue to develop resistant sublines to interesting new drugs, to determine the mechanism of resistance in established sublines and to determine the patterns of cross-resistance to a broad spectrum of cytotoxic agents.

ANNUAL REPORT OF THE LABORATORY OF MEDICINAL CHEMISTRY AND BIOLOGY

DEVELOPMENTAL THERAPEUTICS PROGRAM

NATIONAL CANCER INSTITUTE

July 1, 1976 - September 30, 1977

The Laboratory of Medicinal Chemistry and Biology is a new organizational entity resulting from the consolidation in 1975 of previously established sections from within the Drug Development and Drug Evaluation Branches of the Drug Research and Development Program, DCT; The Laboratory of Chemical Pharmacology, Experimental Therapeutics Program DCT; and the Laboratory of Biochemistry, DCBD. The aim in establishing the new Laboratory was to bring about a closer relationship between medicinal chemists involved in the design and synthesis of new antitumor agents, and biologists involved in the elucidation of their biochemical pharmacology and in their clinical evaluation. It was felt in proposing this new organizational structure that knowledge of the molecular biology and etiology of cancer is approaching the level at which the biologist can now make useful early contributions to the rational design of new antitumor agents, rather than being restricted almost entirely to biochemical, pharmacological and clinical studies on agents whose antineoplastic activity has already been established on an empirical basis. While it is recognized that it would be premature and over-optimistic to propose that empirically-based screening approaches can in the foreseeable future be abondoned in favor of specific, rational antitumor drug design, the time now appears to have arrived when a mixture of empirical and rational approaches is warranted. Examples of drug classes in which opportunities for specific, target-directed design are now possible are agents affecting structure and function of tumor cell surface glycoproteins; inhibitors of viral oncogenesis; agents with specific or unique DNA-binding properties; transition-state inhibitors for key enzyme sites; and agents with specific tissue-localizing properties. In these and a number of other rapidly expanding research areas, the state of the art appears sufficiently advanced that the biologist with specific expertise can make contributions of value to the medicinal chemist in the design of new antitumor agents and in the modification of existing agents.

Conversely, from the point of view of the medicinal chemist, a closer interrelationship with the molecular biologist and the clinician offers the opportunity of obtaining specific biological assay data with the minimum of delay, in order to guide the course of further synthesis. In addition to providing routine antitumor screening, the biologist can design assay methods tailored to the specific target site under consideration. Assays for parameters such as inhibition of viral replication, immunostimulation, enzyme inhibition or tumor cell membrane binding can be provided where relevant to the activity under consideration. Thus, the juxtaposition within a single laboratory of medicinal chemists and biologists from a variety of different disciplines offers the prospect of accelerating drug development, and of supplementing the traditional empirical approaches which have yielded most of our clinically successful antitumor drugs up to the present time with specific target-site directed drug design programs.

The major organizational entities within the new Laboratory of Medicinal Chemistry and Biology, in addition to the Drug Design and Chemistry Section, are the Molecular Biology and Methods Development Section, with the capacity to assess the effects of new agents on cell growth and cell cycle progression; a membrane biology group, with a specific interest in the role of tumor cell membrane alterations in drug resistance; a biochemical pharmacology group concerned with the mode of action of antitumor agents at the molecular level; and an applied pharmacology group with the capacity to carry out drug disposition and metabolic studies in experimental animals and at the Phase I level. The incorporation of the two latter research groups into an Applied Biochemistry Section is planned during the next fiscal year. Some of the individual research projects presently under way in the Laboratory are summarized below.

A major interest of this laboratory has been the modification of the antitumor agent 5-azacytidine, a compound which is of proven value in the treatment of acute myelocytic leukemia, but which has the major practical disadvantage of being highly unstable in aqueous solution, undergoing decomposition in a matter of hours to cleavage products of unknown antitumor activity and toxicity. The most promising candidate to emerge from these studies is 5,6-dihydro-5-azacytidine, prepared by the chemical reduction of 5-azacytidine. This compound has been completely characterized both as the hydrochloride salt (NSC 264880) and the free base (NSC 265483) by gas chromatography, mass spectrometry, N.M.R., U.V. and I.R. spectroscopy and elemental analysis. Acid hydrolysis removed the sugar and produced 5,6-dihydro-5-azacytosine hydrochloride. This compound (NSC 265485) was identical with that produced by the reduction of 5-azacytosine. While solutions of 5-azacytidine in water undergo 25-40% decomposition after 24 hours at room temperature, dihydro-5-azacytidine hydrochloride appears to undergo no decomposition under similar conditions over a period of 60 days.

In addition to stability in aqueous solutions over a broad pH range, the dihydro analogue was found to have a substantial activity in the L1210 leukemia system. In comparison studies, neither drug showed a schedule dependency in the L1210 test system. The QD 1-9 schedule produced a dose-response relationship indicating an optimal dose of 100 mg/kg for the reduced analog while the parent produced the optimal response at 3 mg/kg. Dihydro-5-azacytidine possessed about 80% of the activity shown by the parent at a 30-fold greater optimal dose. The dihydro analog was active against i.p. implanted L1210 when administered orally suggesting a potential use for dihydro-5-azacytidine which is stable in the acidic pH range, as part of a maintenance therapy program. In the treatment of intracerebral L1210 leukemia, the compound exhibited a moderate enhancement (ILS 45%) relative to 5-AC (ILS 28%). However, both drugs were inactive against the murine ependymoblastoma brain tumor model. The dihydro analog showed the same level of activity (ILS 90%) against P388 leukemia as was found in the L1210 system. The solid tumor test system, B16 melanoma, was refractory to both 5-AC and the dihydro derivative.

In in vitro studies, it was observed that both 5-AC and its reduced analog inhibited the growth of L1210 cells in culture, but approximately 10-fold higher concentrations of the dihydro compound were needed for equivalent inhibition. 5-AC at 10 μM completely inhibited the growth of HeLa cells, whereas dihydro-5-azacytidine as high as 200 μM was without effect. In cytidine kinase preparations partially purified from L1210 cells, 5-AC and dihydro-5-AC were phosphorylated at only 5% the rate of cytidine, the natural substrate. 5-AC and the dihydro compound were both substrates for cytidine deaminase partially purified from mouse kidneys or HeLa cells. L1210 cells contained no detectable cytidine deaminase activity. At saturating substrate concentrations, 5-AC was deaminated more readily than either cytidine or dihydro-5-AC. However, the kinetic constants indicated that the binding of the dihydro compound (K_m=29 μM) was close to that of cytidine (K_m=42 μM), whereas the binding of 5-AC was less (K_m=1.8 μM) using the kidney enzyme. Tetrahydrouridine (THU) inhibited the deamination of cytidine, 5-AC and dihydro-5-AC by cytidine deaminase preparations in vitro. However, in HeLa cell cultures, THU potentiated the cytotoxic action of the dihydro compound but not 5-AC.

In order to ensure sufficient quantities of the dihydro analog for preclinical pharmacology studies, a more efficient synthesis was developed which reduced the number of synthetic steps from three to two with an overall two-fold yield improvement.

These studies with dihydro-5-azacytidine have been extended to the synthesis and biological testing of several related compounds of potential antitumor activity. Among the most interesting of these is the arabinosyl analog of 5-azacytidine. As a result of the observed clinical activity of 5-azacytidine (5-AC), the arabinosyl derivative was suggested a number of years ago as an analogue of both 5-AC and cytosine arabinoside. However, a report by R. K. Robins in 1970, which described unsuccessful synthetic attempts to prepare the arabinoside, demonstrated that a straight-forward synthetic scheme would not secure this interesting nucleoside. We have now found that starting with the known 2,3,5-tri-O-benzyl-1-β-D-arabinofuranosyl-5-azacytosine, catalytic hydrogenation gives 5,6-dihydro-1-β-D-arabinofuranosyl-5-azacytosine. By using a novel oxidation procedure, wherein the pertrimethylsilyl derivative undergoes a thermal elimination, the arabinoside has been synthesized in good yield. Both arabinofuranosyl-5-azacytosine (NSC 281272) and its dihydro analog (NSC 270925) are being evaluated in the L1210 system. Initial L1210 tests have shown significant activity for NSC 281272.

In other studies in this area, the two major hydrolysis products of 5-azacytidine have been identified as 1-β-D-ribofuranosyl-3-guanyl-urea and N-(formylamidino)-N'-β-D-ribofuranosylurea. Both compounds have been isolated in useful amounts and the latter has been found to show antitumor activity similar to that of its parent compound, 5-azacytidine.

The Laboratory is pursuing an active interest in the synthesis of organ-specific antitumor agents based on modification of chemical structures from other areas of pharmacology which are known to localize in specific tissues. This program has led to the synthesis of spirohydantoin mustard (NSC 172112) and the diaminodiaziridinylbenzoquinone derivative AZQ (NSC 182986), both of which have recently been selected for DN2 status as potential CNS antitumor agents. Synthesis work with these two series has been completed, but pharmacology and molecular biology studies are presently under way with both compounds, both within LMCB and also in collaboration with other intramural laboratories (Laboratory of Chemical Pharmacology, and the Baltimore Cancer Research Center). Another project of current interest in this area is the synthesis of propanolol mustard as a potential lung tumor agent. The last step of our three step synthetic sequence leading to the mustard requires a selective chlorination of a triol intermediate. Although the selective reaction to date has not been successful, the trichloro compound has been synthesized and will be screened for antitumor activity.

Other synthetic projects currently in progress are the preparation of derivatives of the Lewis lung tumor-active compound usnic acid, and the synthesis of analogues of 3-acetoxy-2-pyridone. The latter compound was found to possess moderate L1210 and P388 leukemia activity in the DR&DP screen. Taking this compound as a lead, a number of analogues have been prepared and tested against the P388 tumor model in an attempt to increase activity and understand the structure-activity relationships in this series. Eight confirmed active analogues were discovered. The 3-hydroxy and 3-amino compounds are both active but the 3-acetylamino derivative is not. Activity in the acyloxy series is abolished by replacement of the acetyl group with bulky lipophilic acyl groups. Activity is retained, however, after N-methylation. Position isomers and 4-pyridones are currently under investigation.

Another major interest of this Laboratory is the development of quantitative structure:activity correlations in the antitumor drug area. These studies are carried out both within our own laboratories and via a contract with Prof. Corwin Hansch of Pomona College. Computer techniques are used to analyze enzyme inhibition and in vivo screening data. These data are then correlated with molecular structural parameters using computerized regression analysis techniques. The potential use of such correlations is to generate leads for new synthesis, to increase the rationale for selection of new compounds to be tested, and to reduce the number of synthetic analogs necessary to obtain the optimally active member of a new series. Contract-supported structure: activity studies have now been completed on the terephthalanilides, aniline mustards, adriamycin analogues, and phenyltriazenes. Intramurally, studies have been carried out on the correlation between structure of heterocyclic thiosemicarbazones and their ability to inhibit the enzyme ribonucleotide reductase and on the development of new biological parameters for the Hansch equation.

A recent development of great interest to this laboratory has been the development within the pharmaceutical industry of several classes of potent inhibitors of the mammalian enzyme adenosine deaminase. A large number of presently available adenosine analogs exhibit antitumor, antiviral and antibacterial activity in in vitro model systems, but their usefulness in vivo has been limited by rapid enzymic deamination and consequent inactivation. The co-administration of an effective adenosine deaminase inhibitor would offer the possibility of preventing this inactivation and thus of potentiating the activity of these analogs. Studies have been initiated within this laboratory with the combination of the adenosine deaminase inhibitors 2'-deoxycoformycin and erythro-9-(2-hydroxy-3-nonyl)-adenine (EHNA) with a number of adenosine analogues; this project is being carried out jointly with the Laboratory of Chemical Pharmacology. Against P388 cells in vitro significant potentiation by 2'-deoxycoformycin of the cytotoxic activity of seven such adenosine analogues has been observed, these observations have been extended to in vivo test systems with two of these analogs: cordycepin (3'-deoxyadenosine and 9-β-xylofuranosyladenine. In vivo studies with the remaining analogues will be carried out when adequate supplies become available.

In addition an enzyme inhibition titration assay method has been developed to measure levels of 2'-deoxycoformycin in biological fluids at drug concentrations as low as 0.2 nanomolar. Pharmacological studies with 2'-deoxycoformycin in experimental animals are well advanced.

Another aspect of our ongoing studies with 2'-deoxycoformycin is the elucidation of the effects of this agent on immune function is experimental animals and also in human peripheral blood lymphocytes. Previous investigators have shown that the enzyme, adenosine deaminase, appears to be important for normal immunologic function. Lymphocytes responding to antigenic stimulation have high specific activity of the enzyme, and lymphocytes from patients born with a deficiency of the enzyme fail to perform normally in vivo or in vitro. Normal lymphocytes can be made to behave abnormally in vitro as though they were ADA deficient by incubating them in the presence of inhibitors of ADA and excess adenosine.

In preliminary studies, we have examined mice which were transplanted with an allograft tumor across an H-2 histocompatibility barrier and were treated with 2'dCF or erythro-2-(2-hydroxy-3-nonyl)-adenosine (EHNA). Mice pretreated with 2'dCF or treated daily with 2'dCF failed to reject their allografts and died of progressive tumor, whereas untreated animals rejected the allograft tumors and survived. This effect could not be demonstrated for EHNA given at several dose levels and by several schedules of administration. These studies with 2'-deoxycoformycin are presently being extended to other model test systems of immune responsiveness. Because of the absence of cytotoxicity of 2'-deoxycoformycin toward other cell types, the inhibition of lymphocyte adenosine deaminase activity by this agent could be a highly specific immunosuppressive strategy.

Studies are continuing on the mode of action of the ansa macrolide maytansine, a plant product which was isolated and characterized by Kupchan and his co-workers in 1972, and which is now in early clinical trial. In studies recently completed in this laboratory, maytansine was found to impair microtubule formation in mammalian cells and to produce metaphase arrest similar to that seen with the Vinca alkaloids. Topics of present investigation are the nature of the maytansine binding site, electron microscopic studies of the alterations in microtubular structure produced by the drug, and the development of bioassay methods for maytansine. It has been found by electron microscope (EM) studies that, in L1210 cells grown in suspension culture and treated with maytansine, there is virtually a complete loss of all microtubules. In untreated mitotic cells, microtubules are well formed and well delineated. It has also been found by EM analysis that the antimitotic effects of maytansine are similar to those seen in parallel cultures treated with colcemid, vincristine sulfate and griseofulvin, although maytansine is more potent.

In studies with microtubular protein from mammalian brain, we have found that maytansine competes for the same binding site as that utilized by the Vinca alkaloids; the binding, however, is considerably tighter than that seen with the reference compound, vincristine. Studies are presently under way to delineate more clearly the mode of binding of maytansine to purified brain tubulin, to study the correlation between tubulin-binding capacity and antitumor activity of a series of maytansine analogs; and to adapt the competitive binding of vincristine and maytansine at the same binding site as a method of bioassay for the latter compound.

Active work has continued on the development of procedures to potentiate the activity and selectivity of antitumor agents by means of pharmacological and nutritional intervention. Approaches to this goal include: 1) alteration of the nutritional status of tumor cells, both in culture and in vivo, to produce conditions interfering with the metabolic inactivation of the chemotherapeutic agents. This approach is particularly directed against resistant cell lines; 2) modification of the cell membrane of susceptible and resistant varieties of tumor cells by the use of either available or newly synthesized membrane-reactive agents; 3) synthesis of analogs of known agents in order to introduce modifications to make them more suitable for the regimens described above.

Of particular promise in the latter area have been our recent studies on the relationship between the membrane transport and the cytotoxicity of the clinically important antitumor agent melphalan (phenylalanine mustard; L-PAM). Melphalan cytotoxicity to murine L1210 leukemia cells in culture is reduced in growth medium containing amino acids. Clonal growth studies indicated that the L-isomers of leucine and glutamine are principally responsible for reducing cytotoxicity. This decrease in cytotoxicity is accompanied by a corresponding decrease in melphalan transport into leucine protected cells. Kinetic analysis of the leucine-melphalan interaction indicates that although melphalan and leucine share a common transport system their affinities differ (leucine K_m=5-10 μM; melphalan K_m=35-50 μM). These results indicate that melphalan cytotoxicity is dependent upon transport by a high-affinity, carrier-mediated, leucine-preferring amino acid transport system.

Promotion of both melphalan cytotoxicity and transport in leucine protected cells by the basic amino acid L-arginine and its homologs occurs by trans-stimulation. There is an increase in cytotoxicity and transport in leucine protected cells with decreasing carbon chain length; L-α-amino-γ-guanidino butyric acid >L-arginine >L-homoarginine. The protection of cells by leucine has been extended to studies in vivo where it has been found that simultaneous administration of leucine interferes with the activity of a therapeutic dose of melphalan. Becuase of their clinical implications, these studies on melphalan transport and cytotoxicity are presently being extended to melphalan-sensitive and resistant human ovarian carcinoma cells in short-term culture; the latter studies are being carried out in collaboration with Medicine Branch CO, DCT.

In attempts to potentiate or modify the cytotoxicity of the clinically important alkylating agent chlorambucil, we have investigated the effect of p-chlorophenoxyacetic acid (CPA), the active moiety of centrophenoxine, and p-chlorophenoxyisobutyric acid (CPB), the active moiety of clofibrate, on the cytotoxicity of chlorambucil in tissue culture. Reports by others have indicated that centrophenoxine, a psycho-active agent, and clofibrate, a blood lipid lowering drug, potentiate the chotherapeutic activity of several alkylating agents. Clonal growth studies in this laboratory indicate that populations of L1210 cells treated with chlorambucil (40 μM) and CPA or CPB (400 μM) for 30 minutes sustained over 10 times the kill of cells incubated with chlorambucil alone. Ultrafiltration experiments with chlorambucil-^{14}C revealed that these concentrations of CPA or CPB doubled the concentration of free chlorambucil in the tissue culture medium and that the level of cell kill was correlated with the amount of free alkylating agent.

Another classical displacer, phenylbutazone, has been shown to potentiate the cytotoxic activity of chlorambucil in vitro and its chemotherapeutic activity in vivo. In the latter case a suboptimal dose of the alkylating agent is significantly potentiated to be equivalent to that of an optimal dose. These studies were complicated by toxicity of the displacing agent to the host, and current studies are directed toward finding effective displacing and potentiating agents in vitro which are not toxic to mice.

A long-term interest of this laboratory has been the pharmacology of the folate antagonists, and in particular, of the widely used agent methotrexate. Last year, we demonstrated the presence in the urine of human subjects and of monkeys which had received high-dose MTX of a metabolite, 7-hydroxyMTX, which is not seen after the administration of MTX at conventional dose levels. Although 7-hydroxymethotrexate is some two orders of magnitude less effective as an inhibitor of dihydrofolate reductase than MTX and does not contribute significantly to the folate antagonist activity of MTX, it may be of significant renal toxicological importance because of its limited aqueous solubility. The presence of this metabolite has now been confirmed by several other laboratories interested in the clinical pharmacology of high-dose MTX.

More recently, we have obtained unequivocal evidence, by means of field desorption mass spectrometry, as to the structure of the 7-hydroxy metabolite, and have also developed a method for the rapid quantitation of this compound in urine by means of high pressure liquid chromatography. We have also supplied reference samples of 7-hydroxyMTX to several other clinical pharmacology laboratories, and one of these (University of Southern California) has developed an HPLC method for the determination of plasma levels of the compound. We have therefore reached the level of technical development to enable us to determine whether a correlation exists between the generation of the 7-hydroxy metabolite and the renal toxicity of high-dose methotrexate.

In other studies with folate antagonists, we have demonstrated in rodents that aminopterin administration, like MTX administration, leads to the formation of the corresponding γ-diglutamate in liver and some other tissues; no γ-diglutamate formation could be demonstrated, however, with 3',5'-dichloroMTX. These studies were carried out because of the recent revival of interest in clinical use of aminopterin and dichloroMTX. We have also carried out biochemical and pharmacological studies on synthetic methotrexate-γ-aspartate and aminopterin-γ-diglutamate generously furnished by Dr. John Montgomery of Southern Research Institute.

A research group has been set up within the Laboratory to study the effects of new and established anticancer agents on the synthesis of biological macromolecules involved in cell replication, i.e., on the translation of messenger RNA, on the synthesis and processing of nuclear RNA, and on the phosphorylation of nonhistone chromosomal proteins. In view of the recent finding within this Laboratory of the potentiation of the in vivo effects of cordycepin (3'-deoxyadenosine) by the adenosine deaminase inhibitor 2'-deoxycoformycin, the effects of cordycepin and other adenosine analogs known to influence these systems are being reexamined under conditions where the catabolism of these agents is blocked by the use of such inhibitors.

The Molecular Biology and Methods Development Section of the Laboratory of Medicinal Chemistry and Biology formerly conducted both an intramural and a contract-supported research program whose objective was the characterization of the mode of action of new drugs at the molecular level. Studies were not restricted to agents under development within LMCB, but encompassed agents being developed by the Developmental Therapeutics Program as a whole. With the promotion of the former Head of this Section (Dr. Vincent Bono) to assume the direction of the Investigational Drug Branch, the contract-supported program has been transferred to IDB. The intramural research program, however, remains within LMCB under the joint direction of the Chiefs of LMCB and IDB. Efforts have continued intramurally on the development of techniques for the analysis of drug effects on proliferative cell cycle progression and for the application of such techniques to the characterization of cancer chemotherapeutic drugs and to the identification of new agents with potential therapeutic activity. The methods employed in this project are mammalian cell culture techniques as applied to the growth of tumor cells in suspension culture; flow microfluorometric determination of cell DNA content of fluorescent-stained cells; and processing, analyzing and plotting of cell DNA content frequency distributions by computer. During the current year, 16 drugs of interest were evaluated for their effects on L1210 cell cycle progression: Cain's Acridine (NSC 141549), Tritylcysteine (NSC 83265), Ellipticine (NSC 71795), Coralyne (NSC 154891), Inox (NSC 118994), Adenox (NSC 266754D), Hycanthone (NSC 142982), "Tetrakis" (NSC 185954D), deoxyuridine-5-thiocyanate (NSC 159861), deoxyuridine-5-propynyloxy (NSC 267459), lapachol (NSC 11905), dichlorolapachol (NSC 126771), "Michigan protein"(NSC 174951), α-picolinic acid, Bouvardia ternifolia (NSC 227262), and Chartreusin (NSC 5159).

The preceding report summarizes the objectives of the new Laboratory of Medicinal Chemistry and Biology, and outlines some of the research carried out within the Laboratory during the year. The individual project reports which follow describe this research in greater detail, and list the publications which have resulted from these studies.

Publications of the Laboratory of Medicinal Chemistry and Biology, 1976-77.

1. Beisler, J.A., Abbasi, M.M. and Driscoll, J.S.: Dihydro-5-azacytidine hydrochloride. A biologically active and chemically stable analog of 5-azacytidine. Cancer Treatment Rep. 60: 1671-1674, 1976.

2. Beisler, J.A., Abbasi, M.M., Kelley, J.A. and Driscoll, J.S.: Synthesis and antitumor activity of dihydro-5-azacytidine. A hydrolytically stable analogue of 5-azacytidine. J. Medicinal Chem., in press.

3. Beisler, J.A., Peng, G.W. and Driscoll, J.S.: Potential antitumor agents. Procarbazine analogs and other methylhydrazine derivatives. J. Pharmaceutical Sci., in press.

4. Bono, V.H. Jr. and Dion, R.L.: Cytotoxic profiles of cell cycle progression. In: Growth kinetics and biochemical regulation of normal and malignant cells. 29th Annual M.D. Anderson Symposium on Fundamental Cancer Research. Baltimore, Williams & Wilkins Co., 1977,pp. 719-732.

5. Chassin, M.M., Chirigos, M.A., Johns, D.G. and Adamson, R.H.: Adenosine deaminase inhibitors for immunosuppression. New England J. Med. 296: 1232, 1977.

6. Chou, F., Khan, A.H. and Driscoll, J.S.: Potential CNS antitumor agents. Aziridinylbenzoquinones II. J. Medicinal Chemistry 19: 1302-1308, 1976.

7. Coronetti, E. and Lippman, M.M.: Increased immunogenicity of TA3Ha cells treated with the antitumor antibiotic macromomycin (B). Journal of the Nat'l Cancer Inst. 56: 1275-1277, 1976.

8. Driscoll, J.S.: Antineoplastic Agents. In Clarke, F.H. (Ed.): Annual Reports in Medicinal Chemistry Vol. 11, New York, Academic Press, 1976 pp. 110-120.

9. Driscoll, J.S. and Beisler, J.A.: Antineoplastic Agents. In Clarke, F.H. (Ed.): Annual Reports in Medicinal Chemistry Vol. 12, New York, Academic Press, 1977, in press.

10. Futterman, B., Derr, C.J., Beisler, J.A., Abbasi, M.M. and Voytek, P.: Studies on the cytostatic action, phosphorylation and deamination of 5-azacytidine and 5,6-dihydro-5-azacytidine in HeLa cells. Biochemical Pharmacology, 1977 (in press).

11. Hirata, T. and Driscoll, J.S.: Potential central nervous system antitumor agents. Phenothiazines I. Nitrogen mustard derivatives. J. Pharma. Sci. 65: 1699-1701, 1976.

12. Hirata, T., Peng, G. and Driscoll, J.S.: Potential central nervous system antitumor agents V. Phenothiazines II. Fluphenazine analogues. J. Pharmaceutical Sci., in press.

13. Jacobs, S.A. and Johns, D.G.: Accumulation of methotrexate diglutamate in human liver during methotrexate therapy. Biochemical Pharmacology 26: 1977 (in press).

14. Jacobs, S.A., Stoller, R.G., Chabner, B.A., Johns, D.G.: Dose-dependent metabolism of methotrexate in man and Rhesus monkey. Cancer Treatment Rep. 61: 1977 (in press).

15. Johns, D.G. and Adamson, R.H.: Enhancement of the biological activity of cordycepin (3'-deoxyadenosine) by the adenosine deaminase inhibitor 2'-deoxycoformycin. Biochemical Pharmacology 25: 1441-1444, 1976.

16. Johns, D.G. and Bertino, J.R.: Folate antagonists. In Holland, J.F. and Frei, E. (Eds.): Cancer Medicine, 2nd Edition. Philadelphia, Lea & Febiger, 1977 (in press).

17. Johns, D.G., Sieber, S.M. and Adamson, R.H.: Studies on the antitumor activity of tilorone. In Chirigos, M.A. (Ed.): Modulation of Host Immune Resistance in the Prevention or Treatment of Induced Neoplasias, Washington, U.S. Government Printing Office, 1977, pp. 117-121.

18. Lippman, M.M. and Mathews, M.B.: Heparins: Varying effects on cell proliferation *in vitro* and lack of correlation with anticoagulant activity. Fed. Proc. 36: 55-59, 1977.

19. Mandelbaum-Shavit, F., Wolpert-DeFilippes, M.K. and Johns, D.G.: Binding of maytansine to rat brain tubulin. Biochem. Biophys. Res. Commun. 72: 47-54, 1976.

20. Okada, K., Kelley, J.A. and Driscoll, J.S.: Intramolecular cyclizations leading to bridgehead bicyclics 2. 5,5-Dialkylhydantoin derivatives. J. Heterocyclic Chem., in press.

21. Okada, K., Kelley, J.A. and Driscoll, J.S.: Intramolecular cyclizations leading to bridgehead bicyclics 3. 5,5-Dipehenyl-2-thiohydantoin derivatives. J. Organic Chem., in press.

22. Schiffman, F.J., Fisher, J.M. and Rabinovitz, M.: Ergosterol-conferred sensitivity to amphotericin B in cultured L1210 mouse leukemia cells. Biochem. Pharmacol. 26: 177-180, 1977.

23. Schiffman, F.J., Fisher, J.M. and Rabinovtiz, M.: Serum displacing agents in cancer chemotherapy. Cancer Treatment Reports, 1977 (in press).

24. Schiffman, F.J., Schiffman, R.L. and Rosa, R.M.: Cellular proliferation and hypokalemia. Annals of Internal Medicine, 1977 (in press).

25. Schiffman, F.J. and Vistica, D.T.: Membrane manipulation and cancer chemotherapy. Lancet 1: 141, 1977.

26. Szekerke, M. and Driscoll, J.S.: The use of macromolecules as carriers of antitumor drugs. European J. Cancer, in press.

27. Vistica, D.T., Toal, J.N. and Rabinovitz, M.: Amino acid conferred resistance to melphalan. I. Structure-activity relationship in cultured L1210 leukemia cells. Cancer Treat. Rep. 60: 1363-1367, 1976.

28. Vistica, D.T., Toal, J.N. and Rabinovitz, M.: Amino acid conferred resistance to melphalan. Characterization of melphalan transport and cytotoxicity in cultured L1210 murine leukemia cells. Cancer Research 1977 (in press).

29. Voytek, P., Beisler, J.A., Abbasi, M.M. and Wolpert-DeFilippes, M.K.: Comparative studies of the cytostatic action and metabolism of 5-azacytidine and 5,6-dihydro-5-azacytidine. Cancer Research 37: 1956-1961, 1977.

The following publications describing collaborative studies with other laboratories also appeared during the period July 1, 1976 - September 30, 1977.

1. Adamson, R.H., Zaharevitz, D.W., and Johns, D.G.: Enhancement of the biological activity of adenosine analogs by the adenosine deaminase inhibitor 2'deoxycoformycin. Pharmacology 15: 84-89, 1977.

2. Chabner, B.A. and Johns, D.G.: Folate antagonists. In Becker, F.F. (Ed.): Cancer, A Comprehensive Treatise, Vol. 5. New York, Plenum Press, 1977, pp. 363-377.

3. Cooney, D.A., Driscoll, J.S., Milman, H.A., Jayaram, H.N. and Davis, R.D.: Inhibitors of L-asparagine synthetase in vitro. Cancer Treat. Rep. 60: 1493-1557, 1976.

4. Sieber, S.M., Wolpert, M.K., Adamson, R.H., Cysyk, R.L., Bono, V.H. Jr., and Johns, D.G.: Experimental studies with maytansine-a new antitumor agent. In Clemmesen, J. and Yohn, D.S. (Eds.): Comparative Leukemia Research, Basel, Karger, 1976, pp. 495-500.

5. Venditti, J.M. and Wolpert-DeFilippes, M.K.: The activity of new drugs against mouse tumors. Cancer Chemotherapy Vol. 7, New York, Plenum Press 1976, pp. 129-147.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 07102-02 LMCB

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Pharmacology of Maytansine, Dimethylbenzylrifampicin, and other Ansa Macrolides

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Mary K. Wolpert, Ph.D.	Pharmacologist	DEB, NCI
Other:	David G. Johns, Ph.D.	Acting Chief	LMCB, NCI

COOPERATING UNITS (if any)

Drug Evaluation Branch, NCI

LAB/BRANCH
Laboratory of Medicinal Chemistry and Biology

SECTION
Office of the Chief

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.5	1.0	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

It is the purpose of this project to study the mode of action and the pharmacology of antitumor agents of the ansa macrolide class. Attention is presently focused on the plant product maytansine and its naturally occurring and semisynthetic derivatives. Maytansine was found to produce metaphase arrest in a manner similar to that seen with the Vinca alkaloids, and to impair microtubule formation in cultured L1210 cells. Topics of current interest are a) the nature of the maytansine binding site; b) a comparison of the binding properties of maytansine with those of its structural analogs; and c) bioassay methods for maytansine.

PHS-6040
(Rev. 10-76)

OBJECTIVES:

The purpose of this project was to study the binding of maytansine to microtubular protein, to ascertain whether maytansine shares a common tubulin binding site with Vincristine. These studies were prompted by the similarities in biological activities between the two agents. Both cause metaphase arrest of cultured L1210 cells; according to electron microscopic analysis of L1210 cells, both block the formation of the microtubules of the mitotic spindle apparatus; and both are ineffective in vivo against a P388 mouse cell line with acquired resistance to Vincristine.

METHODS:

Rat brains were homogenized in 10 mM sodium phosphate buffer (pH 6.5) containing 100 mM sodium glutamate. The homogenate was then centrifuged at 100,000 x g for 45 min at 4°C, and the supernatant was used as the source of tubulin, the major protein component of microtubules. The binding assay (1 ml) consisted of brain protein (1 mg) in 10 mM sodium phosphate buffer (pH 6.5) containing 100 mM sodium glutamate or sodium chloride. Samples were incubated at 37°C in the presence of ^{3}H-Vincristine or ^{3}H-maytansine. The drug-tubulin complex was adsorbed onto buffer-moistened DEAE-cellulose filters (Whatman DE 81) and then filtered under mild suction. The filters were washed with cold buffer, dried and counted using liquid scintillation techniques.

MAJOR FINDINGS:

1. Maytansine binds to crude rat brain tubulin according to a temperature and ionic strength-dependent process.

2. The K_a binding constants are 0.5×10^6 M^{-1} for Vincristine and 1.5×10^6 M^{-1} for maytansine.

3. Maytansine competitively inhibits Vincristine binding with a Ki for maytansine of 0.4×10^{-6} M.

4. The binding of both drugs is easily reversible.

5. Colchicine did not affect the bidning of either Vincristine or maytansine.

SIGNIFICANCE:

The finding that maytansine shares a common binding site with Vincristine suggest that its mechanism of action in inhibiting the polymerization of microtubular protein and disrupting microtubules is similar to that of Vincristine, even at a molecular level. Colchicine, which is also a mitotic inhibitor, appears to be acting at a binding site distinct from that of Vincristine and maytansine. These findings suggest that maytansine, which is now undergoing Phase I clinical trials, may have similar properties in vivo to Vincristine, an agent which is commonly used in the treatment of human cancer.

PROPOSED COURSE:

It is proposed to attempt to study the competition between maytansine and Vincristine using highly purified preparations of tubulin, and to ascertain whether structural congeners of maytansine also bind to tubulin at the same site. The finding that maytansine and Vincristine share a common binding site suggests that it may be possible to measure maytansine levels by means of a competitive binding assay. The possibilities of using this assay to determine maytansine equivalents in biological fluids is under investigation. Such an assay would be of value in correlating plasma levels of maytansine with therapeutic response.

PUBLICATIONS:

Mandelbaum-Shavit, F., Wolpert-DeFilippes, M.K. and Johns, D.G.: Binding of maytansine to rat brain tubulin. Biochem. Biophys. Res. Commun., 72: 47-54, 1976.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 07103-02 LMCB

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Studies on the Clinical Pharmacology of Folic Acid Antagonists

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	David G. Johns, Ph.D.	Acting Chief	LMCB NCI
Other:	Samuel A. Jacobs, M.D.	Montefiore Hospital and University of Pittsburgh,Pittsburgh,Pa.	
	Michael Przybylski	Visiting Fellow	LCP NCI

COOPERATING UNITS (if any)
Laboratory of Chemical Pharmacology, DTP, NCI
Montefiore Hospital, University of Pittsburgh,Pittsburgh,Pa.

LAB/BRANCH
Laboratory of Medicinal Chemistry and Biology

SECTION
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INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.2	0.2	

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

While methotrexate has been in clinical use for a number of years as an antitumor agent, the recent introduction of high dose schedules followed by citrovorum factor rescue in the treatment of solid tumors has resulted in renewed interest in this agent, and has revealed that our knowledge of the clinical pharmacology of this and related folate antagonists is still incomplete. Topics of present interest are: 1) the role of the metabolite 7-hydroxymethotrexate in the renal toxicity frequently observed with high dose methotrexate; 2) metabolic handling of the folate antagonist aminopterin when administered at high dose levels.

PHS-6040
(Rev. 10-76)

Studies have continued with the 7-hydroxyMTX metabolite generated after the administration of high-dose MTX to man and other primates. Further lots of the metabolite have been prepared biologically, by isolation and purification from the urine of Rhesus monkeys given high-dose MTX by slow infusion. Numerous reference samples of 7-hydroxyMTX have been shipped in response to requests from investigators with an interest in high-dose MTX therapy and clinical pharmacology, both in the United States and in Europe. Unequivocal evidence has been obtained by field desorption mass spectrometry of the structure of 7-hydroxyMTX. A high pressure liquid chromatographic and analytical method has been developed for separating and quantitating 7-hydroxyMTX in biological samples which also contain MTX.

Work with MTX polyglutamates has also continued. Biochemical and pharmacological studies have been carried out with diglutamate MTX-γ-aspartate synthesized by Dr. John Montgomery of Southern Research Institute. The compound is as effective an inhibitor of dihydrofolate reductase as is MTX or MTX diglutamate. In vivo, it is much more resistant than is MTX-diglutanate to serum peptidase activity, and much of the drug is excreted unchanged in the urine. Chromatograms of L1210 cell and liver extracts revealed, however, that intracellularly, the compound was hydrolyzed quantitatively to MTX. Antitumor activity in the L1210 murine leukemia test system was almost identical to that seen with MTX, as would be anticipated from the biochemical data. It appears, however, that Dr. Montgomery and his colleagues have been successful in preparing an MTX diglutamate which is more resistant to hydrolysis than is MTX-γ-glutamate, and which can thus act as a slow-release form of MTX.

Because of the recent clinical interest in high-dose aminopterin and high-dose 3',5'-dichloroMTX (with citrovorum rescue), we have determined whether these two agents, like MTX, yield a diglutamate form intracellularly. Aminopterin-γ-glutamate has been detected in rat liver after administration of aminopterin, but no diglutamate form has been detected after dichloroMTX.

Proposed Course:

We hope to apply the HPLC method described above for quantitation of 7-hydroxyMTX to clinical urine and plasma samples, to ascertain whether any correlation exists between rate of formation of this metabolite and MTX renal toxicity in man. Because of the current interest in high-dose aminopterin, studies will be carried out in Rhesus monkeys to determine whether this compound, like MTX, yields a 7-hydroxy metabolite when given in a high-dose regimen. The question has clinical relevance, since one of the reasons for substituting high-dose aminopterin for high-dose MTX is the prospect of avoiding the renal toxicity frequently seen as a consequence of high-dose therapy with the latter drug.

Publications:

Jacobs, S.A. and Johns, D.G.: Accumulation of methotrexate diglutamate in human liver during methotrexate therapy. Biochemical Pharmacology, 26: 1977 (in press).

Jacobs, S.A., Stoller, R.G., Chabner, B.A. and Johns, D.G.: Dose-dependent metabolism of methotrexate in man and rhesus monkeys. Cancer Treatment Reports, 61: 1977 (in press).

Chabner, B.A. and Johns, D.G.: Folate antagonists. In Becker, F.F. (Ed.): Cancer, A Comprehensive Treatise, Vol. 5. New York, Plenum Press, 1977, pp. 363-377.

Johns, D.G. and Bertino, J.R.: Folate antagonists. In Holland, J.F. and Frei, E. (Eds): Cancer Medicine, 2nd Edition. Philadelphia, Lea & Febiger, 1977 (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 07104-02 LMCB

PERIOD COVERED

July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Nutritional and Pharmacological Regimens for Potentiation of Chemotherapeutic Activities.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Marco Rabinovitz, Ph.D.	Research Chemist	LMCB	NCI
Other:	David T. Vistica, Ph.D.	Research Associate	LMCB	NCI
	Fred J. Schiffman, M.D.	Research Associate	LMCB	NCI
	Yoshimasa Uehara, Ph.D.	Visiting Fellow	LMCB	NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Medicinal Chemistry and Biology

SECTION

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INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS: 5.7	PROFESSIONAL: 3.5	OTHER: 2.2

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The goal of this project is to develop procedures which will potentiate the activity of current and novel cancer chemotherapeutic agents. Binding, transport, cell membrane composition and agent detoxification are modified in an attempt to direct the active agent to susceptible tumor cells. Investigations are initiated in tissue culture and when applicable are extended to tumor bearing mice. Studies include 1) inhibition kinetics of melphalan transport, where it was found that cytotoxicity of this alkylating agent to the L1210 leukemia cell depends upon its uptake by a high-affinity, leucine-preferring, amino acid transport system; 2) competitive binding studies with chlorambucil, where agents which compete with this alkylating agent for serum binding can improve cytotoxicity by increasing the availability of free drug and 3) alteration of tumor cell membrane composition, where pretreatment of cells with ergosterol potentiates the cytotoxic action of amphotericin-B.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION:

A. Alkylating Agent Studies

Melphalan cytotoxicity to murine L1210 leukemia cells in culture is reduced in growth medium containing amino acids. Clonal growth studies indicated that the L-isomers of leucine and glutamine are principally responsible for reducing cytotoxicity under conditions where the cells had been preincubated in PBS-0.1 mM BSA containing these amino acids. This decrease in cytotoxicity is accompanied by a corresponding decrease in melphalan-^{14}C transport into leucine protected cells. Kinetic analysis of the leucine-melphalan interaction indicates that although melphalan and leucine share a common transport system their affinities differ (leucine K_m = 5-10 μM; melphalan K_m = 35-50 μM). These results indicate that melphalan cytotoxicity is dependent upon transport by a high-affinity, carrier-mediated, leucine-preferring amino acid transport system.

Promotion of both melphalan cytotoxicity and transport in leucine protected cells by the basic amino acid L-arginine and its homologs occurs by trans-stimulation. There is an increase in cytotoxicity and transport in leucine protected cells with decreasing carbon chain length; L-α-amino-γ-guanidino butyric acid >L-arginine >L-homoarginine.

The protection of cells by leucine has been extended to studies in vivo where it has been found that simultaneous administration of leucine interferes with the activity of a therapeutic dose of melphalan. Since melphalan is given by mouth clinically, this indicates that the accompanying diet may influence its activity.

Melphalan is transported by another system at concentrations several fold higher than that needed to kill L1210 cells. The possible interplay between the two transport systems in determining the relation between chemotherapeutic sensitivity and resistance and host toxicity will be the subject of continuing investigations.

Reports by others have indicated that centrophenoxine, a psychoactive agent, and clofibrate, a blood lipid lowering drug, potentiate the chemotherapeutic activity of several alkylating agents. We have investigated the effect of p-chlorophenoxyacetic acid (CPA), the active moiety of centrophenoxine, and p-chlorophenoxyisobutyric acid (CPB), the active moiety of clofibrate, on the cytotoxicity of chlorambucil in tissue culture. Clonal growth studies indicated that populations of L1210 cells treated with chlorambucil (40 μM) and CPA or CPB (400 μM) for 30 minutes sustained over 10 times the kill of cells incubated with chlorambucil alone. Ultrafiltration experiments with chlorambucil-^{14}C revealed that these concentrations of CPA or CPB doubled the concentration of free chlorambucil in the tissue culture medium and that the level of cell kill was correlated with the amount of free alkylating agent.

Another classical displacer, phenylbutazone, has been shown to potentiate the cytotoxic activity of chlorambucil in vitro and its chemotherapeutic activity in vivo. In the latter case a suboptimal dose of the alkylating agent is significantly potentiated to be equivalent to that of an optimal dose. These studies were complicated by toxicity of the displacing agent to the host, and current studies are directed toward finding effective displacing and potentiating agents in vitro which are not toxic to mice.

B. Membrane Modification Studies

L1210 cells which had been previously incubated in ergosterol are more selectively destroyed in vitro by amphotericin B than cells not treated with this sterol. This observation has been extended to a combined in vitro - in vivo condition; amphotericin-B is more effective in prolonging the life of mice bearing P388 cells previously incubated in vitro with ergosterol than of mice bearing cells incubated with cholesterol as a control. Further investigations to optimize ergosterol delivery to tumor cells are needed before complete in vivo trials are undertaken.

PUBLICATIONS:

Vistica, D. T., Toal, J. N. and Rabinovitz, M.: Amino acid conferred resistance to melphalan. I. Structure-activity relationship in cultured L1210 leukemia cells. Cancer Treat. Rep. 60: 1363-1367, 1976.

Schiffman, F. J., Fisher, J. M. and Rabinovitz, M.: Ergosterol-conferred sensitivity to amphotericin B in cultured L1210 mouse leukemia cells. Biochem. Pharmacol. 26: 177-180, 1977.

Schiffman, F. J. and Vistica, D. T.: Membrane manipulation and cancer chemotherapy. Lancet 1: 141, 1977.

Schiffman, F. J., Schiffman, R. L. and Rosa, R. M.: Cellular proliferation and hypokalemia. Annals of Internal Medicine, 1977 (in press).

Vistica, D. T., Toal, J. N. and Rabinovitz, M.: Amino acid conferred resistance to melphalan. Characterization of melphalan transport and cytotoxicity in cultured L1210 murine leukemia cells. Cancer Research, 1977 (in press).

Schiffman, F. J., Fisher, J. M. and Rabinovitz, M.: Serum displacing agents in cancer chemotherapy. Cancer Treatment Reports, 1977 (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 07105-02 LMCB

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)
Biochemical and Pharmacological Studies of Pyrimidine Nucleoside Analogs with Antitumor Activity

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Peter Voytek, Ph.D.	Staff Fellow	LMCB	NCI
Other:	David G. Johns, Ph.D.	Acting Chief	LMCB	NCI
	John S. Driscoll, Ph.D.	Chemist	LMCB	NCI
	Mary K. Wolpert, Ph.D.	Pharmacologist	DEB	NCI

COOPERATING UNITS (if any)
Drug Evaluation Branch, NCI

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NCI, NIH, Bethesda, Maryland 20014

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1.5	1.0	0.5

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The goal of this project is to study the pharmacologic activity and the biochemical mode of action of new antitumor agents of the pyrimidine nucleoside class. Present studies are focused on 5-azacytidine and 5,6-dihydro-5-azacytidine, a reduced analog of the latter with greater chemical stability than the parent compound.

OBJECTIVES:

The major objective of this project was to study the mode of action of 5-azacytidine (5-AC) in comparison with a chemically more stable derivative, 5,6-dihydro-5-azacytidine (DHAC), in a variety of experimental systems.

METHODS:

The effect of 5-AC and DHAC on the growth of L1210 and HeLa cells in culture was determined. The conversion of both 5-AC and DHAC to their respective monophosphates was examined using cytidine kinase preparations partially purified from L1210 or HeLa cells, according to a procedure described by Furlong. Phosphorylation of C^{14}-labeled cytidine, 5-AC and DHAC was also compared in L1210 cell preparations. Deamination of 5-AC, DHAC and cytidine by cytidine deaminase partially purified from mouse kidneys or HeLa cells was compared using a coupled assay to glutamate dehydrogenase.

MAJOR FINDINGS:

1. Both 5-AC and its analog DHAC inhibited the growth of L1210 cells in culture, but approximately 10-fold higher concentrations of DHAC were needed for equivalent inhibition. 5-AC at 10 μM completely inhibited the growth of HeLa cells, whereas DHAC as high as 200 μM was without effect.

2. Cytidine and uridine were able to protect cultured L1210 cells from the growth inhibitory effect of 5-AC and DHAC, while thymidine potentiated the cytotoxic effect of both.

3. L1210 cell suspensions phosphorylated cytidine, 5-AC and DHAC according to the following rates: 24, 15 and 2 nmoles nucleotides/10^9 cells/hr.

4. In cytidine kinase preparations partially purified from L1210 cells, 5-AC and DHAC were phosphorylated at only 5% the rate of cytidine, the natural substrate.

5. 5-AC and DHAC were both substrates for cytidine deaminase partially purified from mouse kidneys or HeLa cells. L1210 cells contained no detectable cytidine deaminase activity. At saturating substrate concentrations, 5-AC was deaminated more readily than either cytidine or DHAC. However, the kinetic constants indicated that with the kidney enzyme, the binding of DHAC (Km=29 μM) was close to that of cytidine (Km=42 μM), whereas the binding of 5-AC was significantly less (Km=1.8 μM).

6. Tetrahydrouridine (THU) inhibited the deamination of cytidine, 5-AC and DHAC by cytidine deaminase preparations *in vitro*.

7. In HeLa cell cultures, THU potentiated the cytotoxic action of DHAC, but not 5-AC.

SIGNIFICANCE:

Although DHAC is less potent, it is similar in biological properties to the parent drug 5-AC. Our results showing a reduced rate of phosphorylation of DHAC in L1210 cells as compared to 5-AC may explain its lack of potency. It is possible that DHAC does not permeate cells as well as the parent, since in cell-free preparations the differences in the rates of phosphorylation between the two compounds were less marked.

PROPOSED COURSE:

The finding that the cytotoxic effect of DHAC against HeLa cells could be enhanced by the addition of THU will be investigated in tumor-bearing animals. The 5-AC-thymidine combination, will also be examined further in tissue culture and in whole animals, in an attempt to improve the therapeutic efficacy of 5-AC and its analogs.

PUBLICATIONS:

Voytek, P., Beisler, J. A., Abbasi, M. M., and Wolpert-DeFilippes, M.K.: Comparative studies of the cytostatic action and metabolism of 5-azacytidine and 5,6-dihydro-5-azacytidine. Cancer Research, 37: 1956-1961, 1977.

Futterman, B., Derr, C. J., Beisler, J. A., Abbasi, M. M. and Voytek, P.: Studies on the cytostatic action, phosphorylation and deamination of 5-azacytidine and 5,6-dihydro-5-azacytidine in HeLa cells. Biochemical Pharmacology, 1977 (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 07106-02 LMCB

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Adenosine Deaminase Inhibitors as Potentiators of the Pharmacological Activity of Adenosine Analogs

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	David G. Johns, Ph.D.	Acting Chief	LMCB	NCI
Other:	Marvin M. Chassin, M.D.	Clinical Associate	LMCB	NCI
	Daniel W. Zaharevitz	Chemist	LMCB	NCI
	Richard H. Adamson	Chief	LCP	NCI

COOPERATING UNITS (if any)

Laboratory of Chemical Pharmacology, DTP, NCI

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3.0	2.5	0.5

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

A number of presently available adenosine analogs possess antitumor activity. Their usefulness has been limited by their rapid metabolism by adenosine deaminase. 2'Deoxycoformycin (2'dCF), a powerful inhibitor of adenosine deaminase has been able to potentiate the antitumor activity of some adenosine analogs in vitro and in vivo. The purpose of this work was (1) extend those observations to other adenosine analogs and (2) to study the pharmacokinetics of 2'deoxycoformycin and its in vivo effects on adenosine deaminase.

PHS-6040
(Rev. 10-76)

Earlier studies from this laboratory have shown 2'dCF to potentiate the antitumor effect of cordycepin in vivo and in vitro. In new studies, we examined the effects of 6 additional adenosine analogs - 8-azaadenosine, 6-chloropurine ribonucleoside, formycin A, 4'-thioadenosine, tubercidin, and xylosyl adenine with and without 2'dCF on growth of P388 leukemia cells in suspension culture. All agents exhibited variable growth inhibition at concentrations of 0.01 to 1 μg/ml. There was striking enhancement of inhibition with the same analog concentrations in the presence of 1 μg/ml 2'dCF for all compounds except tubercidin which is not a substrate for adenosine deaminase. 9-β-D-Xylofuranosyladenine (xylosyladenine) was then selected for study in vivo. CDF_1 mice inoculated with P388 ascites leukemia were treated daily x 9 with xylosyladenine with and without 2'dCF 0.25 mg/kg/d. Without 2'dCF, optimal survival (50% ILS) was obtained using 50 mg/kg of xylosyladenine. 2'dCF alone was without therapeutic effect. In combination, the optimal dose of xylosyladenine was 3.1 mg/kg, median survival time was increased to 250% of control and there were 20% long term survivors.

The kinetics of the six adenosine analogs as substrates for P388 adenosine deaminase was also studied. Tubercidin was found not to be a substrate for the enzyme. Formycin A and 8-azaadenosine have the highest K_m values and are deaminated faster than the natural substrate adenosine. All other analogs are deaminated less rapidly.

The pharmacokinetics of 2'dCF were studied in foxhound dogs. Plasma disappearance curves are biphasic with $t_{\frac{1}{2}}$ of 12-15 min and 90-120 min, respectively. CSF levels peak at 2^o post iv injection and are 10% of plasma levels. 55-85% of the drug is recovered in urine within 6^o. The drug is concentrated with respect to plasma in all tissues examined, most significantly in lung.

Tissue adenosine deaminase levels post treatment are not solely a function of 2'dCF level, but are also related to the rate of enzyme recovery, possibly by new enzyme synthesis.

Further studies of 2'dCF and its effect on adenosine deaminase levels in primates will be carried out in conjunction with toxicology studies of the drug.

PUBLICATIONS:

Adamson, R. H., Zaharevitz, D. W., and Johns, D. G.: Enhancement of the biological activity of adenosine analogs by the adenosine deaminase inhibitor 2'deoxycoformycin. Pharmacology 15: 84-89, 1977.

Johns, D. G. and Adamson, R. H.: Enhancement of the biological activity of cordycepin (3'-deoxyadenosine) by the adenosine deaminase inhibitor 2'-deoxycoformycin. Biochemical Pharmacology 25: 1441-1444, 1976.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 07107-02 LMCB

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Biological Studies on Metastatic and Non-Metastatic Melanomas

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Theodore R. Breitman, Ph.D.	Research Chemist	LMCB NCI
Other:	G. H. Sato	Prof., Biology Dept	Univ. of Calif.

COOPERATING UNITS (if any)

Department of Biology, University of California, San Diego, California

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The growth of three human melanoma cell lines, BRL 4143, BRL 6005 and BRL 6278 was enhanced by the addition of insulin, transferrin, NIH.FSH, NIH.LH, and progesterone to a nutritional medium containing only 1% fetal bovine serum. The results indicate that melanomas require hormones for growth.

PHS-6040
(Rev. 10-76)

Objectives:

To define the growth requirements of established human melanoma cell lines with particular emphasis on defining the factors and hormones in the serum component of the medium.

Major Findings:

Three established human melanoma cell lines have been studied. These cell lines were obtained from Dr. Grace Cannon of Bionetics Research Laboratory who established them from metastatic lesions. These cell lines require the addition of approximately 10% serum to the nutritional medium for maximal growth. By decreasing the serum concentration and replacing it with hormones we have been able to grow one cell line, BRL 6278, in the presence of only 1% serum. The other two cell lines respond but not to the same extent. The hormones or factors requried were: insulin, transferrin, progesterone, LRF, and NIH.FSH.

Additional evidence that melanomas are responsive to hormones comes from work with nude mice. Tumor takes and growth rate were greater in female than in male mice. This finding takes on a greater interest since the three melanoma cell lines originated from female patients.

We have also attempted to establish in culture many of the human transplantable tumors that are being carried on at the University of California, San Diego, nude mouse colony. One tumor that was of particular interest was a melanoma that in the mouse was slightly pigmented. Since most, if not all, of the human melanoma cell lines are amelanotic we felt it would be of great utility to establish a highly pigmented transplantable human melanoma and then to establish this tumor in culture.

By the laborious and time consuming process of dissecting out highly pigmented portions of the melanoma, mincing, and injection into nude mice we have over the last year been able to develop a highly melanotic melanoma. This melanoma has now been established in culture. It is in its 14th subculture and it forms tumors in nude mice.

Significance to Biomedical Research and Proposed Course:

The results present above, coupled with my results summarized in the 1976 annual report, indicate that melanomas are hormone dependent tumors. This should have obvious chemotherapeutic potential.

These studies should be continued with additional melanoma cell lines, especially those from human males.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 07108-01 LMCB

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)
Adenosine Deaminase Inhibitors as Immunosuppressive Agents

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Marvin M. Chassin, M.D.	Clinical Associate	LMCB	NCI
Other:	David G. Johns, M.D.,Ph.D.	Acting Chief	LMCB	NCI
	Richard H. Adamson, Ph.D.	Chief	LCP	NCI
	Michael Chirigos, Ph.D.	Head, Virus and Disease Modification Section	LRNATV	NCI

COOPERATING UNITS (if any)
LRNATV, Viral Oncology Program, DCCP, NCI

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1.5	1.0	0.5

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☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of these studies is to examine the effect of 2'-deoxycoformycin and other potent inhibitors of adenosine deaminase on in vitro parameters of human peripheral blood lymphocytic function, and on in vivo parameters of immune function in experimental animals.

PHS-6040
(Rev. 10-76)

The enzyme, adenosine deaminase (ADA), appears to be important for normal immunologic function. Lymphocytes responding to antigenic stimulation have high specific activity of the enzyme, and lymphocytes from patients born with a deficiency of the enzyme fail to perform normally *in vivo* or *in vitro*. Normal lymphocytes can be made to behave abnormally *in vitro* as though they were ADA deficient by incubating them in the presence of inhibitors of ADA and excess adenosine.

The purpose of the present studies is (1) to examine the effect of 2'-deoxycoformycin (2'-dCF), a potent inhibitor of ADA, on *in vitro* parameters of of human peripheral blood lymphocyte function and (2) to examine the effects of 2'-dCF on *in vivo* parameters of immune function in animals.

Human peripheral blood lymphocytes were stimulated to undergo blast transformation by the nonspecific mitogen Conconavalin A. This transformation was inhibited by 100 μM Adenosine, and synergistically inhibited by 2'-dCF at 10^{-6} and 10^{-7} M in combination with adenosine.

Mice were transplanted with an allograft tumor across an H-2 histocompatibility barrier and the animals were treated with 2'-dCF or erythro-2-(2-hydroxy-3-nonyl)-adenosine (EHNA). Mice pretreated with 2'-dCF, or treated daily with 2'-dCF failed to reject their allografts and died of progressive tumor, whereas untreated animals rejected the allograft tumors and survived. This effect could not be demonstrated for EHNA given at several dose levels and by several schedules of administration.

These findings support the importance of adenosine deaminase for some functions of the normal immune response and suggest that pharmacologic inhibition of adenosine deaminase activity may be an effective immunosuppressive strategy.

These studies are being continued using other models of immune responsiveness including skin graft rejection, immunoglobulin synthesis and delayed hypersensitivity skin test response. Also lymphocytes taken from animals being treated with 2'-dCF are being examined *in vitro* for effects on their function.

Publications:

Chassin, M.M., Chirigos, M.A., Johns, D.G. and Adamson, R.H.: Adenosine deaminase inhibitors for immunosuppression. *New England J. Med.* 296: 1232, 1977.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 07109-01 LMCB

PERIOD COVERED
February 1, 1977 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Effect of Anticancer Drugs on the Synthesis and Processing of Nuclear RNA

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Robert I. Glazer, Ph.D.	Pharmacologist	LMCB	NCI
Other:	Debra Davis	Chemist	LMCB	NCI

COOPERATING UNITS (if any)

None

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0.6	0.3	0.3

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The synthesis and processing of nuclear ribosomal RNA (rRNA) and heterogeneous RNA (hnRNA) will be studied in vivo in partially hepatectomized rats and in vitro in L1210 tumor cells. [^{3}H]-Orotic acid and [^{3}H]-uridine will be used to pulse label and measure the synthesis of various species of nuclear RNA in rat liver and L1210, respectively. The methylation of rRNA and hnRNA, as well as the polyadenylation of hnRNA will be examined as representative post-transcriptional processes. The nucleoside antibiotic, cordycepin (3'-deoxyadenosine), will be used to examine its specific inhibitory action on rRNA and poly(A)- and poly(A)+hnRNA in the presence and absence of the adenosine deaminase inhibitor, 2'-deoxycoformycin.

PHS-6040
(Rev. 10-76)

OBJECTIVES:

Many anticancer drugs act by inhibiting the synthesis of mRNA via interference with the activity of either RNA polymerases or the DNA template. Nucleolar RNA polymerase I and nucleoplasmic RNA polymerase II transcribe the 45S precursor to rRNA and the heterogeneous RNA (hnRNA) precursor to mRNA, respectively. These two species of RNA undergo further modification or processing via 1) polyadenylylation of the 3' terminus and methylation of internal adenine as well as 5' terminal guanine and ribose in hnRNA, and 2) methylation of ribose in 45S rRNA. These unique features confer biochemical specificity for the nucleolytic trimming of the rRNA precursor, as well as recognition features for the transport from the nucleus and binding to the 40S subunit of the ribosomes for mRNA. The objectives of this project are to characterize the inhibitory activity of anticancer agents, viz. nucleosides, on the synthesis and processing of rRNA and hnRNA. Analysis of the sensitivity of these biochemical processes to anticancer drugs will allow an assessment of their possible role as future biochemical targets for the design of new chemotherapeutic agents.

METHODS EMPLOYED:

Isolation of nuclei from regenerating liver and L1210 by ultracentrifugation in hypertonic sucrose; incorporation of [^{3}H] orotic acid and [^{3}H] uridine into mRNA from liver and L1210, respectively. Incorporation of [^{3}H-methyl]-methionine into ribose and nucleotides of mRNA; differential pH extraction of rRNA with SDS-sodium acetate-NaCl (pH 5.1) and phenol mixture (phenol: cresol:water, 7:2:2 v/v, containing 0.1% 8-hydroxyquinoline) and hnRNA with SDS-Tris-urea (pH 9.0) and phenol mixture; isolation of poly(A) hnRNA by poly(U)Sepharose affinity chromatography; isolation of the [^{3}H-methyl] 5' cap of hnRNA after alkaline hydrolysis by affinity chromatography on dihydroxyboronylphenylamino-Sepharose; analysis of N^{6}methyladenosine monophosphate and 2'-O-methylribosemonophosphate by urea-DEAE-Sephadex chromatography; agarose-urea gel electrophoresis of rRNA and hnRNA; assay of adenosine deaminase spectrophotometrically at 305 nm with formycin A as substrate.

MAJOR FINDINGS:

1) Effect of 2'-deoxycoformycin on adenosine deaminase levels in normal and regenerating liver. In order to study the effect of the adenosine deaminase inhibitor, 2'-deoxycoformycin (2'dCF), on the action of cordycepin (3'-deoxyadenosine) on mRNA synthesis, a determination of the dose and time of treatment for 2'dCF was made. 2'dCF rapidly inhibited adenosine deaminase within one min after i.p. injection of 0.063 mg/kg. Inhibition was dose-dependent with an ID_{50} (10 min) of 0.007 mg/kg and 0.006 mg/kg for normal and 18 hr regenerating liver, respectively.

2) Effect of cordycepin and 2'dCF on mRNA synthesis. Cordycepin produced a dose-dependent inhibition of nuclear rRNA (ID_{50} = 15 mg/kg), non-poly(A) hnRNA (ID_{50} = 20 mg/kg) and poly(A)hnRNA (ID_{50} - 15 mg/kg) within 45 min using a pulse labeling time of 15 min for [^{3}H]-orotic acid (500 μCi/kg). Pretreatment for 10 min with 0.063 mg/kg of 2'dCF resulted in only a small potentiation of the effect of cordycepin on rRNA (ID_{50} = 10 mg/kg), non-poly(A)hnRNA (ID_{50} = 15 mg/kg) and poly(A)hnRNA (ID_{50} = 10 mg/kg) synthesis. Either larger doses of 2'dCF (up to 1 mg/kg) or longer pretreatment times or both, did not increase the degree of potentiation by 2'dCF. Either tubercidin or Ara-A at doses of 25 mg/kg in the presence or absence of 2'dCF were without effect on mRNA synthesis in 18 hr regenerating liver. mRNA synthesis in normal liver was not affected by cordycepin at doses up to 25 mg/kg in the presence or absence of 2'dCF.

PROPOSED COURSE:

Characterization of the aforementioned experimental protocol used in regenerating liver will be carried out in vitro using L1210. Since this tumor is sensitive to cordycepin in the presence of 2'dCF, probably by virtue of its high level of adenosine deaminase (10-fold greater than liver), it should allow an assessment of the differential sensitivity of various species of mRNA to cordycepin and the role of adenosine deamination in the action of this nucleoside anticancer drug, as well as of other adenosine analogs with potential antineoplastic activity. Further analysis of the processing steps for rRNA and hnRNA and their relevance to the action of cordycepin and other nucleoside anticancer drugs will be carried out.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 07110-01 LMCB

PERIOD COVERED
June 1, 1977 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Effect of Anticancer Drugs on the Translation of Messenger RNA in vitro

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Robert I. Glazer, Ph.D.	Pharmacologist	LMCB	NCI
Other:	Chris Carrico, Ph.D.	Research Associate	LMCB	NCI

COOPERATING UNITS (if any)

None

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0.2	0.2	

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Antimetabolites such as 5-fluorouracil and 5-azacytidine are believed to exert antitumor effects through their incorporation into mRNA, and hence interference with the ability of mRNA to code for proteins critical to the balanced growth of the cell. This hypothesis will be tested by quantitative and qualitative assessment of the capacity of L1210 poly(A)mRNA from drug-treated tumor-bearing mice to code for proteins in an in vitro heterologous translation system. These studies will include an analysis of both the 5'cap and 3'-poly(A) structures of mRNA, as well as the synthesis of the mRNA in vivo. This experimental system will allow an assessment of the effect of anticancer drugs on the synthesis, post-transcriptional processing and coding properties of tumor mRNA.

OBJECTIVES:

Many antimetabolites such as 5-fluorouracil and 5-azacytidine are believed to exert their antitumor effects, at least in part, by being incorporated into mRNA and initiating miscoding during translation. To test this hypothesis, poly(A)RNA from polyribosomes of rat liver or L1210 will be isolated and analyzed in an *in vitro* translation system prepared from wheat germ embryos. The labeled translation products will be analyzed by polyacrylamide gel electrophoresis. In addition, poly(A)RNA will be analyzed for chemical modification of its 3'- and 5'-termini, as well as its rate of synthesis resulting from treatment with anticancer drugs.

METHODS EMPLOYED:

Isolation of polyribosomes by Mg^{++} precipitation of 10,000 x g extracts of rat liver or L1210. Extraction of poly(A)RNA by SDS-phenol-$HCCl_3$ and poly(U) Sepharose chromatography. Preparation of 23,000 x g extracts of wheat germ embryos stored in liquid nitrogen. Incubation of poly(A)RNA with $[^3H]$ leucine or $[^{35}S]$methionine, amino acids, KCl GTP, ATP, PEP, Mg^{++}, spermidine and wheat germ extract. Extraction of labeled proteins and analysis on slab or cylindrical polyacrylamide gels. Analysis of poly(A) levels by its template activity with $(dT)_{10}$ and E. coli DNA polymerase A. Measurement of the 5'cap, m^7GpppXmpXmp, by dihydroxyboronylphenylamino Sephadex chromatography.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 07111-01 LMCB

PERIOD COVERED
February 28, 1977 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Action of Anticancer Drugs on Phosphorylation of Nonhistone Chromosomal Proteins

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Robert I. Glazer, Ph.D.	Pharmacologist	LMCB	NCI
Other:	Michel LeGraverend, Ph.D.	Visiting Fellow	LMCB	NCI

COOPERATING UNITS (if any)

None

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The phosphorylation of nonhistone chromosomal proteins (NHCP) appears to exert a regulatory influence on transcription in eukaryotic cells. The recent discovery that cordycepin (3'-deoxyadenosine) can inhibit normal and neoplastic nuclear protein kinase activities in vitro has prompted an investigation of the role of phosphorylation in the action of this and related drugs. The phosphorylation of NHCP in vitro in isolated nuclei will be used to study the effect that cordycepin has on various classes of NHCP as determined by isoelectric focusing. These studies will be extended to L1210 cells in vitro to correlate the effects of cordycepin on nuclear phosphorylation and RNA synthesis. The latter system will also allow a determination of the effect that the adenosine deaminase inhibitor, 2'-deoxycoformycin, has on the action mediated by cordycepin on the phosphorylation of NHCP.

PHS-6040
(Rev. 10-76)

OBJECTIVES:

The phosphorylation of nonhistone chromosomal proteins (NHCP) is believed to exert a regulatory role in transcription in eukaryotic cells. This process is mediated via nuclear protein kinases, a heterogeneous group of isoenzymes which are cyclic nucleotide independent. Cordycepin (3'-deoxyadenosine) competitively inhibits protein kinase activities partially purified from rat liver or hepatoma 3924A. The objectives of this project are: 1) to characterize the NHCP which are phosphorylated in vitro in isolated nuclei in the presence and absence of cordycepin, and 2) investigate the effect of cordycepin on the phosphorylation of NHCP in vitro in L1210 cells. The two aspects of this project will provide information about the mode of action of cordycepin in isolated nuclei and intact cells on the phosphorylation of NHCP, and the relevance of this effect to its inhibitory action on RNA synthesis.

METHODS EMPLOYED:

Isolation of nuclei from rat liver or L1210 by ultracentrifugation in hypertonic sucrose. Labeling of NHCP in nuclei in incubation under optimal conditions with [γ^{32}P]ATP. Extraction of NHCP with 7 M urea-phosphate buffer. Characterization of NHCP by isolectric focusing using 0.5 x 6.5 cm 5% polyacrylamide gels with pH 3.5-10 ampholytes. Purification of [^{32}P]-nuclear proteins by QAE-Sephadex chromatography prior to isoelectric focusing.

MAJOR FINDINGS:

Several discrete urea-extractable NHCP were visualized from rat liver nuclei by isolectric focusing for 15 hr at 150V constant voltage. Proteins at pH = 5, 5.5, 6, 6.5 and 7.0 were apparent with the greatest ^{32}P - labeling occurring in a sharp major peak at pH 7.0. [^{32}P]-Proteins solubilized from nuclei were chromatographed on QAE-Sephadex. The peak coinciding with RNA polymerase I was highly labeled with ^{32}P. The pattern visualized by isoelectric focusing of this fraction revealed several bands similar to those of the urea-extractable NHCP. Radioactivity was again concentrated in the proteins focusing at pH 7.0. Cordycepin reduced the labeling of all NHCP with [γ^{32}P]ATP, with the largest percentage reduction in the proteins with a pI of 7.0. The effect of cordycepin in isolated nuclei in vitro was competitive.

PROPOSED COURSE:

The action of cordycepin will be extended to L1210 cells in vitro to better explore the significance of inhibition of phosphorylation of NHCP in whole cells. This system will also enable an analysis of the effect of adenosine deaminase inhibitors on the activity of cordycepin and other adenosine analogs.

Drug Design and Chemistry Section

Laboratory of Medicinal Chemistry and Biology

July 1, 1976 - September 30, 1977

Scope

The objectives of the Drug Design and Chemistry Section of the Laboratory of Medicinal Chemistry and Biology are: (1) chemical research in the design and synthesis of new drugs for the treatment of cancer, (2) the chemistry of agents and drugs of interest in the treatment of cancer, (3) research on the structure, purity, decomposition products and metabolites of antitumor drugs by analytical instrumental techniques, (4) the development of structure-activity techniques to summarize existing data and to provide guidelines for analog synthesis and (5) the promotion of the interdisciplinary approach to the synthesis and evaluation of rationally designed drugs by collaboration between chemical and biological scientists.

Staff

The staff consisted of three scientists, one Senior Staff Fellow, one NCI Expert, one NIH Visiting Scientist, five NIH Visiting Fellows, one secretary and one College Work-Study technician.

Summary of Accomplishments

Approximately ten man-years of effort were devoted to intramural research projects described in Individual Project Reports Z01-CM-03580, Z01-CM-03581, and Z01-CM-03582 during the fifteen month period covered. Two agents developed somewhat earlier in these laboratories, AZQ (NSC 182986) and dihydro-5-azacytidine (NSC 264880), were selected as DN2 compounds during this period.

Significant research was carried out on 5-azacytidine (5-AC). The rate of decomposition of this material under clinical infusion conditions was determined. Even more importantly, the major solution decomposition product of 5-AC was isolated, identified, characterized and evaluated biologically. The previously unobtainable arabinosyl analogue of 5-azacytidine was synthesized utilizing a novel oxidation system discovered during the research leading to dihydro-5-azacytidine (DH5-AC). DH5-AC was further characterized with respect to its chemical oxidation reactions and its mode of action in several enzymatic systems. An improved synthesis of DH5-AC was also developed.

In the area of target-specific agents, potential lung tumor agents were emphasized. A nitrogen mustard analogue of propranolol was prepared as were several derivatives of usnic acid, a Lewis lung active compound. As the result of the exploitation of a screening lead, eight 2-pyridone derivatives were synthesized which possessed confirmed P388 leukemia activity.

Mass spectrometric, gas chromatographic and high pressure liquid chromatographic methods were devised to identify, isolate and quantitate a number of new triazine nucleosides and their reduction and oxidation products, propranolol derivatives, hydantoins, pyridones, phenothiazines and usnic acid derivatives. A computerized data system was chosen for our mass spectrometer and made operational. This data system allows the mass spectrometer to be used in its most efficient manner in both the high and low resolution modes.

Section personnel were also involved in the extramural contract research program. They served as project officers on a Pomona College contract which has the objective of correlating chemical structure with antitumor activity in a quantitative manner. Correlations were obtained in the terephthalanilide (Wander), aniline mustard, adriamycin and phenyltriazene series.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 03580-08 LMCB

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Chemical Research in the Development of New Anticancer Drugs

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. S. Driscoll	Head, Drug Design and Chem. Section	LMCB	NCI
OTHER:	J. A. Beisler	Research Chemist	LMCB	NCI
	M. M. Abbasi	Visiting Fellow	LMCB	NCI
	V. Feyns	Visiting Fellow	LMCB	NCI
	D. R. Hwang	Visiting Fellow	LMCB	NCI
	V. E. Marquez	Visiting Scientist	LMCB	NCI
	K. Okada	Visiting Fellow	LMCB	NCI
	G. R. Proctor	NCI Visiting Expert	LMCB	NCI
	M. Takai	Visiting Fellow	LMCB	NCI

COOPERATING UNITS (if any)

Drug Evaluation Branch, DR&DP, DCT, NCI
Lab. Chem. Pharmacology, DR&DP, DCT, NCI

LAB/BRANCH
Laboratory of Medicinal Chemistry & Biology

SECTION
Drug Design and Chemistry

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS: 7.0	PROFESSIONAL: 7.0	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The objective of this project is the discovery of new types of clinically useful anticancer drugs. The following topics are of current interest: (1) nucleosides, reduced nucleosides and the chemistry of 5-azacytidine, (2) target specific agents for CNS and lung tumors, (3) pyridones and phenalkylamines as antitumor agents and (4) transition-state enzyme inhibitors.

Project Description:

General Objective:

The objective of this project is the discovery of new types of clinically useful anticancer drugs. Medicinal chemical research is directed toward the synthesis of new compounds which have potential as useful agents. Leads for this program are generated from structure-activity studies, the DR&DP screening program and the literature.

Specific Objectives:

1. Investigation of the aqueous solution chemistry of 5-azacytidine in order to better understand the toxicity and activity of the decomposition products of this drug under clinical conditions;
2. Synthesis and antitumor activity of triazine nucleosides and their reduced analogues;
3. Design and synthesis of target specific drugs, especially those directed to tumors of the CNS and lung;
4. Exploitation of screening leads in the pyridone and phenethylamine families;
5. Transition-state enzyme inhibitors.

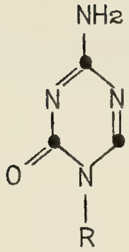
Major Findings:

A. Nucleoside Antimetabolites

1. The Chemistry of 5-Azacytidine (5-AC) (Dr. Beisler)

a. Aqueous decomposition study: To minimize gastrointestinal toxicity, 5-AC (A-1, NSC 102816) is best administered by continuous i.v. infusion over a period of several days. However, this administration method is complicated by the instability of the drug in aqueous formulations. Since an appropriate study of the decomposition under clinical conditions was not available in the literature, an investigation in lactated Ringer's solution at 25° was initiated using high-pressure liquid chromatography as an analytical tool. The disappearance of 5-AC follows a first order kinetic equation wherein an initial rapid loss of drug is followed by a more moderate decomposition rate, i.e. after 3 hr 89% 5-AC remains, after 6 hr 82%, and after 24 hr 63%. The half-life of the drug in this solution is 47 hr. Dilute solutions (0.5 mg/ml, 0.05 mg/ml), typical of those used for continuous infusion administration, decompose at the same rate as the more concentrated solution (5 mg/ml) obtained by reconstituting a clinical vial according to label instructions.

b. Isolation and Biological Study of 5-AC Hydrolysis Products: It is well-known that 5-AC is unstable in aqueous formulations. Little, however, is known concerning how the hydrolysis products contribute to toxic or therapeutic effects in humans. It was found that aqueous solutions of



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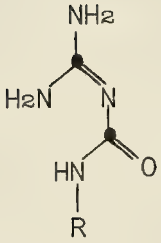

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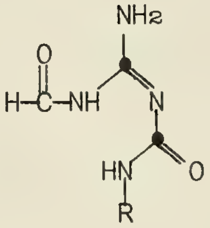

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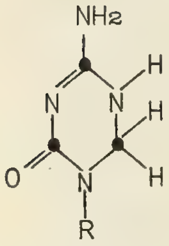

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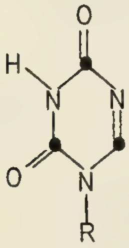

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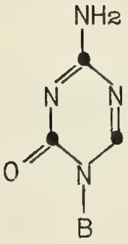

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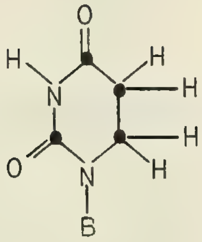
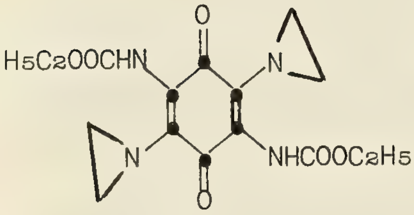
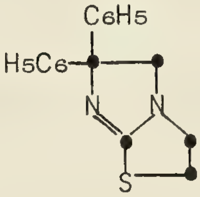


A-7

R = β-D-ribofuranosyl
B = β-D-arabinofuranosyl



B-1



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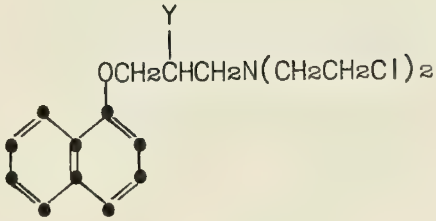

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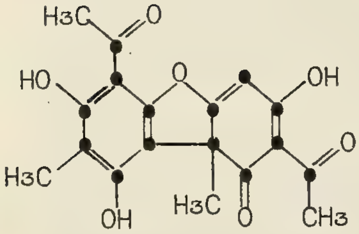
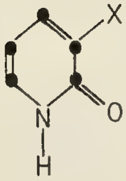
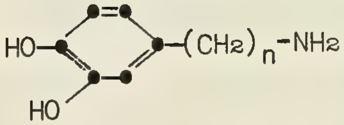


B-4



C-1



C-2

5-azacytidine at neutral pH and room temperature yield two major hydrolysis products: 1-β-D-ribofuranosyl-3-guanylurea (A-2) and N-(formylamidino)-N'-β-D-ribofuranosylurea (A-3). Preparative high-pressure liquid chromatography was used to collect samples of both products, as well as unreacted 5-AC, for identification and study. The known guanylurea (A-2, NSC 264412) was isolated and found to be moderately toxic but inactive in the murine L1210 leukemia system. The formylamidino product (A-3, NSC 279851) had previously eluded all isolation or synthetic preparation attempts. High-pressure liquid chromatography, used in the preparative mode, made possible the isolation of this unstable hydrolysis product in useful amounts and allowed its characterization by UV, IR, NMR and elemental analysis. Preliminary results against in vivo L1210 leukemia indicate an antitumor response for A-3 similar to that obtained with 5-AC.

2. Dihydro-5-azacytidine (Drs. Beisler, Abbasi, Driscoll): 5,6-Dihydro-5-azacytidine hydrochloride (A-4, NSC 264880) was rationally designed and synthesized to provide a solution-stable analogue of the clinical antileukemic agent, 5-AC. In addition to stability in aqueous solutions over a broad pH range, the dihydro analogue, A-4, was found to have a substantial activity in the L1210 leukemia system. In comparison studies, neither drug showed a schedule dependency in the L1210 test system. The QD 1-9 schedule produced a dose-response relationship indicating an optimal dose of 100 mg/kg for the reduced analog (A-4) while the parent (A-1) produced the optimal response at 3 mg/kg. A-4 possessed about 80% of the activity shown by the parent at a 30-fold greater optimal dose. The dihydro analog was active against i.p. implanted L1210 when administered orally suggesting a potential use for A-4, which is stable in the acidic pH range, as part of a maintenance therapy program. In the treatment of intracerebral L1210 leukemia, A-4 exhibited a moderate enhancement (ILS 45%) relative to 5-AC (ILS 28%). However, both drugs were inactive against the murine ependymoblastoma brain tumor model. The dihydro analog showed the same level of activity (ILS 90%) against P388 leukemia as was found in the L1210 system. The solid tumor test system, B16 melanoma, was refractory to both 5-AC and A-4.

It is possible that the reduced analog derives its antileukemic activity from a metabolic conversion to 5-azacytidine since: (a) both are active in the same leukemia test systems but are inactive against the B16 solid tumor; (b) neither has any apparent schedule dependency; (c) in experiments with a L1210/5-AC resistant tumor, a cross resistance was observed. The dihydro analogue can be chemically converted to 5-AC using several oxidizing agents.

Dihydro-5-azacytidine had cytostatic activity against cultured L1210 cells but at a concentration which was 10-fold higher than the parent. The addition of either cytidine or uridine protected against growth inhibition by both 5-AC and A-4. In cell-free extracts, both drugs were phosphorylated and both were found to be substrates for mouse kidney cytidine deaminase. 5-AC inhibited the growth of HeLa cells as did A-4, but only when combined with tetrahydrouridine.

In order to ensure sufficient quantities of the dihydro analog for preclinical pharmacology studies, a more efficient synthesis was developed which reduced the number of synthetic steps from three to two with an overall twofold yield improvement.

3. 5-Azauridine (Drs. Abbasi, Beisler): Biological systems are known to enzymatically deaminate 5-AC to produce 5-azauridine (A-5). It is not known whether the deamination is strictly an inactivation process or if 5-azauridine contributes to the antitumor activity associated with 5-AC. Although Czech workers prepared A-5 about 10 years ago using a lengthy synthesis with a low overall yield, the NCI has never had this compound for evaluation. In order to obtain sufficient amounts of this nucleoside for in vivo and in vitro studies, a synthetic effort was begun to produce 5-azauridine in three synthetic steps or less. Condensation of trimethylsilylated 5-azauracil with β-D-ribofuranose 1-acetate-2,3,5-tribenzoate has produced 5-azauridine tribenzoate in 32% yield. Deblocking this molecule has proven difficult. Future work will concentrate on increasing the yield of the initial condensation step and circumventing the difficulties encountered in the deblocking step.

4. Arabinofuranosyl-5-azacytosine (Drs. Abbasi, Beisler): As a result of the observed clinical activity of 5-azacytidine (5-AC), the arabinosyl derivative (A-6) was suggested a number of years ago as an analogue of both 5-AC and cytosine arabinoside. However, a report by R. K. Robins in 1970, which described unsuccessful synthetic attempts to prepare A-6, demonstrated that a straight-forward synthetic scheme would not secure this interesting nucleoside. It has now been shown that starting with the known 2,3,5-tri-O-benzyl-1-β-D-arabinofuranosyl-5-azacytosine, catalytic hydrogenation gives 5,6-dihydro-1-β-D-arabinofuranosyl-5-azacytosine. By using a novel oxidation procedure, wherein the pertrimethylsilyl derivative undergoes a thermal elimination, A-6 has been synthesized in good yield. Both arabinofuranosyl-5-azacytosine (NSC 281272) and its dihydro analog (NSC 270925) are being evaluated in the L1210 system. Initial L1210 tests have shown significant activity for A-6.

5. Reduced Ara-C and Ara-U Derivatives (Drs. Proctor, Kelley, Driscoll) A study of the reduction products of Ara-C and Ara-U was undertaken with the objective of producing cytidine deaminase inhibitors structurally related to tetrahydrouridine (THU). Two dihydro (NSC 273440, 273441) and one tetrahydro (NSC 275646) acetylated Ara-C derivatives were synthesized and characterized. The 5,6-dihydro Ara-U derivative (A-7, NSC 275645) was also prepared. Testing is underway. The present objective of this study is the synthesis of the tetrahydroarabinofuranosyl analogue of THU.

B. Target Specific Antitumor Agents

1. CNS Antitumor Agents

a. AZQ (Dr. Driscoll): AZQ (B-1, NSC 182986), synthesized as a potential CNS antitumor agent, was selected recently for DN2 status based on its intracerebral (IC) ependymoblastoma and IC L1210 activities. This compound is also active against the intraperitoneal L1210, P388 and B16 melanoma tumor models. Synthesis work has been completed in this area.

b. Aliphatic procarbazine analogs (Dr. Beisler): The synthesis of three aliphatic analogs and derivatives has been completed. Antitumor testing of these materials in the L1210 system gave negative results and no encouragement for further work. This project is considered complete and no additional work is planned.

c. Bicyclic hydantoins and levamisole analogues (Drs. Okada, Driscoll): Several bicylcic thiohydantoins were prepared as possible CNS agents or alkaline phosphatase inhibitors. One of these compounds was converted into the levamisole analogue (B-2). Since neither murine *in vivo* antitumor activity nor enzyme inhibitory activity was found in this series, this project has been terminated.

2. Lung Antitumor Agents

a. Propranolol mustard (Drs. Feyns, Beisler): Dr. Adamson (LCP) suggested propranolol mustard (B-3, Y=OH) as a potential lung tumor agent. The last step of our three step synthetic sequence leading to the mustard requires a selective chlorination of a triol intermediate. Although the selective reaction to date has not been successful, the trichloro compound (B-3, Y=Cl) has been synthesized and will be screened for antitumor activity.

b. Lung specific agents for antitumor evaluation (Dr. Beisler): Of the 25 tuberculosis drugs screened for activity in the i.v. Lewis lung tumor system, none showed activity. This project will be held in abeyance pending fresh insight as to the selection of additional compounds for screening.

c. Usnic acid derivatives (Drs. Takai, Beisler): A survey of all the compounds submitted to the DR&DP screening program with reproducible activity in the Lewis lung tumor system revealed a lichen metabolite, usnic acid (B-4), which showed low level activity in both the Lewis lung and the P388 leukemia test systems. Since usnic acid is commercially available, a program was initiated to synthesize derivatives of the natural material guided by the following rationale: (a) the poor solubility of usnic acid in aqueous solution might be a contributing factor to the observed low biological activity. Since poor solubility can be associated with the existence of several intramolecular hydrogen bonds, disruption of one or more of the internal hydrogen bonds through derivative formation could lead to compounds with enhanced activity; (b) among several possibilities, glycoside derivatives are attractive because they are capable of eliminating internal hydrogen bonds while adding an aqueous solubilizing moiety. Tumors with an elevated glucoronidase activity should allow a glucoronide conjugate of usnic acid to be cleaved more readily in a tumor cell than in normal cells; (c) as derivatives are designed, synthesized and tested, attention is given the development of structure-activity relationships to determine the areas of the usnic acid molecule necessary for activity. Several of the eleven derivatives prepared have shown moderate P388 activity.

C. Development of Screening Leads

1. 2- and 4-Pyridones (Drs. Hwang, Driscoll): 3-Acetoxy-2-pyridone (C-1, X=$OCOCH_3$) was found to possess moderate L1210 and P388 leukemia activity in the DR&DP screen. Taking this compound as a lead, a number of analogues have been prepared and tested against the P388 tumor model in an attempt to increase activity and understand the structure-activity relationships in this series. Eight confirmed active analogues were discovered. The 3-hydroxy and 3-amino compounds are both active but the 3-acetylamino derivative is not. Activity in the acyloxy series is abolished by replacement of the acetyl group with bulky lipophilic acyl groups. Activity is retained, however, after N-methylation. Position isomers and 4-pyridones are currently under investigation.

2. Phenethylamines (Dr. Driscoll): The discovery that dopamine (C-2, n=2) had reproducible L1210 and P388 activity in the "Psychotropic Drug Study" led to the testing of the corresponding benzylamine derivative (C-2, n=1). This compound is also reproducibly active. A program has been devised to discover the structure-activity relationships in this series.

D. Transition State Enzyme Inhibitors (Drs. Marquez, Driscoll)

Plans are underway to establish an interdisciplinary research program in this area. The target enzyme and potential inhibitors will be chosen by consultation among the biochemical and medicinal chemical members of the team.

Publications

Hirata, T. and Driscoll, J.S.: Potential CNS Antitumor Agents. Phenothiazines I. Nitrogen Mustard Derivatives. J. Pharmaceutical Sci., 65: 1699-1701, 1976.

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 03581-08 LMCB

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

The Analytical Chemistry of New Anticancer Drugs

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. A. Kelley	Sr. Staff Fellow	LMCB	NCI
OTHER:	J. S. Driscoll	Head, Drug Design and Chem. Section	LMCB	NCI

COOPERATING UNITS (if any)

Laboratory of Toxicology, DR&DP, DCT, NCI

LAB/BRANCH
Laboratory of Medicinal Chemistry and Biology

SECTION
Drug Design and Chemistry

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.4	1.4	

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The objective of this project is the research and development of analytical techniques which are used to: establish the structure and purity of new antitumor agents and their metabolites, determine physical and chemical properties of new anticancer drugs and study reaction mechanisms. Mass spectrometry, gas-liquid chromatography and the combination of these techniques is emphasized. Compounds of current interest are 5-azacytidine, nucleosides, hydantoins, propranolol mustard, pyridones, usnic acid, phenothiazines and alanosine.

PHS-6040
(Rev. 10-76)

Project Description:

General Objectives:

The objective of this project is the research and development of analytical techniques which are used to:

1. establish the structure and purity of new anticancer drug candidates;
2. establish structures of metabolites of new antitumor agents;
3. determine important physical and chemical properties of new anticancer drugs; and
4. study reaction mechanisms.

Methods Employed:

Mass spectometry (MS), gas-liquid chromatography (GC) and the combination of these techniques (GC/MS) are emphasized. Other analytical methods such as high pressure liquid chromatography (HPLC), NMR, UV and IR spectroscopy also are employed.

Major Findings:

A. Dihydro-5-azacytidine (Dr. Kelley)

GC, GC-MS and MS techniques were employed to characterize synthetic products related to dihydro-5-azacytidine. A gas chromatographic method for qualitatively determining ribosyl and arabinosyl nucleosides was developed using the isothermal retention indices of their trimethylsilyl derivatives on two different liquid phases. This complemented GC-MS procedures where the mass spectra of two isomers were identical.

A mass spectral method was developed for the rapid analysis of products from the hydrogen peroxide oxidation of both 5-azacytidine and dihydro-5-azacytidine. Oxidation of 5-azacytidine resulted in hydroxylation of the 6-position of the triazine ring. Reaction of dihydro-5-azacytidine under similar conditions gave a mixture of 5-azacytidine, its 6-hydroxy derivative, and ammelide, the aglycone of the 6-hydroxy nucleoside.

A quantitative gas chromatographic assay using an internal standard was developed for 5-azacytidine and employed to investigate the scope and kinetics of a novel silylation-mediated oxidation. This reaction dehydrogenated dihydropyrimidine and dihydro-s-triazine bases as well as their respective nucleosides. With dihydro-5-azacytidine, the reaction proceeded smoothly under mild conditions to give 5-azacytidine in quantitative yield. When dihydro-5-azacytidine-d_1 was oxidized, the product showed preferential retention of deuterium at the 6-position of the base with an isotope effect of 2.7. This method may thus be applicable to the synthesis of deuterium or tritium labeled parent drugs from their reduced derivatives. The feasibility of synthesizing 5-azacytosine arabinoside from its dihydro analog was demonstrated by GC-MS studies which were also used to optimize conditions.

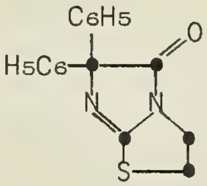
Conversion of the reduced arabinosyl nucleoside also occurred in quantitative yield, but at only 25% of the rate of the ribosyl analog.

B. Arabinosylcytosine (Ara-C) (Drs. Kelley and Proctor)

Analytical methods employing GC, HPLC and GC-MS were developed for the analysis of both crude reaction mixtures and purified products resulting from the controlled catalytic hydrogenation of Ara-C. The parent nucleoside was reduced to 5,6-dihydrocytosine arabinoside which could be trapped as the pentaacetate or rapidly hydrolyzed to 5,6-dihydrouracil arabinoside. Hydrogenation of uracil arabinoside also produced 5,6-dihydrouracil arabinoside. Catalytic reduction of tetraacetyl Ara-C resulted in the formation of a tetrahydrotetraacetate derivative. Current efforts are directed toward the synthesis of an arabinosyl tetrahydrouracil for testing and comparison with tetrahydrouridine as a cytidine deaminase inhibitor.

C. Bicyclothiohydantoins (Dr. Kelley)

The mass spectral fragmentation of isomeric bicyclothiohydantoin derivatives (A and B) and their degradation products was investigated in detail to allow unambiuous structural assignment. A major distinguishing mass spectral feature was the more facile extrusion of the elements of CO and HCO from the 3-substituted hydantoin derivatives than from the corresponding 1-substituted isomers. GC-MS analysis was also employed to identify intermediates and to determine impurities during the preparation of the substituted levamisole (C).



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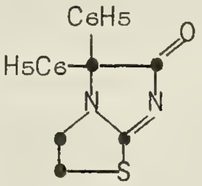
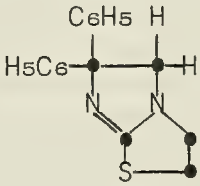


(B)



(C)

D. Propranolol Mustard (Dr. Kelley)

Both gas chromatographic and GC-MS procedures were developed for the analysis of propranolol mustard (see Z01 CM 03580-08 LMCB) and the corresponding trichloride. This assay procedure was used to monitor reaction mixtures, identify by-products and evaluate compound stability.

E. Alanosine (Dr. Kelley and Dr. Cooney, LT)

Attempts are being made to identify a metabolite of alanosine isolated from mouse urine.

F. Synthesis Project Support (Dr. Kelley)

1. Pyridones: Mass spectral analyses of a series of acylated 3-hydroxypyridones indicated that compounds mono-acylated on oxygen thermally decompose

to the starting material, 2,3-dihydroxypyridine, *via* elimination of an isocyanate.

2. *Usnic acid*: A series of methylated diacetyl usnic acid derivatives were examined to determine purity and structure.

3. *Phenothiazines*: Mass spectral analyses of phenothiazine analogues whose elemental analyses indicated incorporation of a fractional mole of water corroborated the proposed structures.

Publications:

Cooney, D.A., Driscoll, J.S., Milman, H.A., Jayaram, H.N. and Davis, R.D.: Inhibitors of L-asparagine synthetase *in vitro*. *Cancer Treat. Rep.*, 60: 1493-1557, 1976.

Okada, K., Kelley, J.A. and Driscoll, J.S.: Intramolecular Cyclizations Leading to Bridgehead Bicyclics 2. 5,5-Dialkylhydantoin Derivatives. *J. Heterocyclic Chem.*, in press.

Okada, K., Kelley, J.A. and Driscoll, J.S.: Intramolecular Cyclizations Leading to Bridgehead Bicyclics 3. 5,5-Diphenyl-2-thiohydantoin Derivatives. *J. Organic Chem.*, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 03582-03 LMCB

PERIOD COVERED

July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Structure-Activity Studies of Anticancer Drugs

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	F. R. Quinn	Chemist	LMCB	NCI
OTHER:	J. S. Driscoll	Head, Drug Design and Chem. Section	LMCB	NCI

COOPERATING UNITS (if any)

Stanford Research Institute
Genesee Corporation
Pomona College
Drug Evaluation Branch, DR&DP, DCT, NCI

LAB/BRANCH

Laboratory of Medicinal Chemistry and Biology

SECTION

Drug Design and Chemistry

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.1	1.1	

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The objectives of this project are: (1) the development of quantitative structure-activity correlations among antitumor agent congeners, (2) the development of general structure-activity relationships among large numbers of unrelated antitumor agents and (3) the use of the correlation data obtained to generate leads for new synthesis and increase the rationale for the testing of new agents. Compound families of current interest are adriamycin, terephthalanilide, thiosemicarbazones, aniline mustards and rifamycin analogues.

PHS-6040
(Rev. 10-76)

Project Description:

General Objectives:

The objectives of this project are:

1. the development of quantitative correlations of screening data with structural features among antitumor agent congeners;
2. the investigation of structure-activity techniques as applied to antitumor data;
3. the development of correlations between structure and antitumor activity among large numbers of structurally unrelated compounds;
4. the use of the correlations obtained to: (a) generate leads for new synthesis, (b) increase the rationale for selection of new compounds to be tested and (c) reduce the number of synthetic analogs necessary to obtain the optimally active member of a new series.

Methods Employed:

Computer techniques are used to analyze and organize biological screening data. These data are then correlated with molecular structural parameters using computerized regression analysis techniques.

Major Findings:

I. Quantitative Structure-Activity Relationships by the Hansch Technique (Dr. Quinn)

A. Intramural Studies

1. Heterocyclic Thiosemicarbazones: A study is underway to correlate molecular structure with enzymatic (nucleoside diphosphate reductase) and *in vivo* activity for this set of molecules.

2. Rifamycin analogs: A cooperative project is underway with Dr. Wolpert to correlate SSV-DNA-polymerase inhibition with molecular structure.

3. New Biological Parameters for the Hansch Equation: A study was undertaken with the aid of a statistical consultant to determine whether a more reliable measure of biological activity than T/C could be extracted from *in vivo* data. The Proportional Hazards Model which utilizes life table techniques was explored and a new survival function is being evaluated.

B. Contract Studies

1. Terephthalanilides: A study of the toxicity (LD_{50}) and L1210 antitumor activity of a series of active terephthalanilides is being completed. Indications are that toxicity is increased by hydrophobic substitution on the amidine binding site and the presence of electron-withdrawing groups on the

central benzene ring. Activity is favored by increased hydrophilicity and the presence of electron-donating substituents on the central phenyl ring.

2. Aniline Mustards: A study of the structure-activity relationship of aniline mustards in the Walker 256, L1210 and P388 tumor models has been completed. It was found that electron-releasing ring substituents increase both antitumor activity and toxicity. Indications are that there is a different ideal log P required for solid tumors relative to leukemias. This is being investigated further.

3. Adriamycin analogs: A cooperative study with Pomona College and the Stanford Research Institute has resulted in a correlation of P388 *in vivo* data for this family of anthracycline antibiotics. The hydrophobic character of these drugs was shown to contribute little to their activity. Acylation of the amino function was found, in general, to be detrimental to activity. A study has been initiated to correlate the B16 melanoma solid tumor activity of these congeners.

4. Phenyltriazenes: A study of phenyltriazenes is being completed. Correlations have been achieved for both toxicity (LD_{10}) and *in vivo* activity (L1210 and P388).

II. Other Correlations

A. Aziridinylbenzoquinones (Drs. Driscoll and Geran)

Analysis of the biological data from 39 members of this family resulted in the choice of NSC 182986 (AZQ) as a DN2 compound.

B. Psychotropic Drugs (Driscoll, Melnick, Quinn, Lomax, Davignon, Ing, Abbott, Congleton and Dudeck)

A selective screening study utilizing compounds with known CNS activity was conducted against the L1210 tumor system. Sixteen L1210 confirmed active compounds were discovered among the 297 screened. This was 18 times the yield which would have been expected from the screening of randomly obtained compounds. Neuroleptic agents (antipsychotic drugs) were the most active class found.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 03579-04 LMCB

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Effect of Chemotherapeutic Agents on L1210 Cell Cycle Progression

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Vincent H. Bono, Jr.	Chief	IDB NCI
Other:	Robert L. Dion	Chemist	LMCB NCI

COOPERATING UNITS (if any)
The Upjohn Company, Kalamazoo, Michigan, Cancer Research Contract N01-CM-43753, Laboratory of Molecular Biology, DCBD, NCI

LAB/BRANCH
Laboratory of Medicinal Chemistry and Biology

SECTION
Molecular Biology and Methods Development Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS: 1 1/4	PROFESSIONAL: 1 1/4	OTHER:

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Project is designed to investigate the effects of cancer chemotherapeutic agents on cell cycle progression based on their cytotoxicity in an in vitro L1210 murine leukemia system. DNA content distribution (DCD) curves are generated with the use of a flow microfluorometer and drug treated curves are compared with steady state DCD curves after analysis by computer. An attempt to classify these agents as to their mode of action (anti-metabolite, etc.) by computer analysis is in progress.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION:

This project is concerned with the development of techniques for the analysis of drug effects on the progress of cells through the proliferative cell cycle and their application to (a) the characterization of therapeutically active drugs and (b) the identification and possible selection of agents with potential therapeutic activity. The major methods employed in this project are (a) mammalian cell culture techniques applied to the growth of L1210 murine leukemia cells in suspension culture, (b) flow microfluorometric determination of cell DNA content using fluorescent hypotonic citrate/propidium iodide stained cells in a Cytofluorograf (Biophysics Systems, Inc.) interfaced with a multichannel pulse height analyzer and (c) processing, analyzing and plotting cell DNA content distributions (DCD) profiles by computer.

During the current year, 16 drugs of interest were evaluated for their effects on L1210 cell cycle progression: Cain's Acridine (NSC-141549), Tritylcysteine (NSC-83265), Ellipticine (NSC-71795), Coralyne (NSC-154891), Inox (NSC-118994), Adenox (NSC-266754D), Hycanthone (NSC-142982), "Tetrakis" (NSC-185954D), deoxyuridine-5-thiocyanate (NSC-159861), deoxyuridine-5-propynyloxy (NSC-267459), lapachol (NSC-11905), dichlorolapachol (NSC-126771), "Michigan protein" (NSC-174951), α-picolinic acid, Bouvardia ternifolia (NSC-227262), and Chartreusin (NSC-5159).

MAJOR FINDINGS:

Inox and Adenox at ED_{50} dose levels appear to block cell progression primarily in G_2 phase. There is a question of whether endoreduplication occurs as results varied according to the staining techniques employed.

"Michigan protein" was analytically separated by gel permeation chromatography in a 20% DMSO/neutral buffer system into a high molecular weight inactive species and a toxic moiety with a molecular weight of 1000-1500. The latter species comprises 1-2% of the bulk material. A single attempt at large scale purification of the active moiety was unsuccessful.

In a collaborative study with Drs. George Johnson and Alberto Fernandez-Pol, α-picolinic acid effects on DNA content distribution curves of normal and transformed rat kidney cells were investigated. Normal cells were reversibly arrested in G_1 phase whereas four lines of virally transformed cells showed responses which were dependent on the type of transforming virus. Kirsten sarcoma virus transformed cells are blocked in G_1. SV40 transformed cells progress to a G_2 block. Cells transformed by polyoma or Harvey sarcoma virus with Moloney virus coat have DCD profiles which indicate blocks in both G_1 and G_2. Cells transformed with Moloney sarcoma virus are not blocked in a specific phase of the cell cycle.

An additional collaborative study on L1210 survival and cell cycle progression was conducted with Dr. Bijoy Bhuyan of the Upjohn Company using the drug Chartreusin (NSC-5159) which binds to DNA. Results showed that in cells in S phase were more sensitive to the drug's toxic effect than cells in other phases of the cycle. Flow microfluorometry (FMF) revealed that effects on cell cycle progression were concentration and exposure time dependent. The DNA content distribution (DCD) curves for cells given a four hour exposure to drug followed by a subsequent four hour recovery in the absence of drug were remarkably similar to continual drug exposure for eight hours. With a 20 hour recovery, DCD curves reverted to essentially untreated control curves whereas continuous exposure resulted in extensive degradation of cellular DNA.

This project relates to the Drug Developmental Program in providing a characterization of the cell biological properties of therapeutically active drugs and in exploring the possibility of developing a new screen for the selection of drugs having therapeutic activity. In addition to continuing the current research effort, it is planned to attempt to develop a method whereby DNA content and synthesis rate of individual cells can be measured simultaneously.

Keywords: Cancer chemotherapeutic drugs, cell cycle progression, cell DNA content, cytotoxicity, endoreduplication, flow microfluorometry, hypotonic propidium iodide.

Publications:

Bono, V.H. Jr. and Dion, R.L.: Cytotoxic profiles of cell cycle progression. In: Growth kinetics and biochemical regulation of normal and malignant cells. 29th Annual M.D. Anderson Symposium on Fundamental Cancer Research. Baltimore, Williams & Wilkins Co., 1977, pp. 719-732.

ANNUAL REPORT OF THE LABORATORY OF MOLECULAR PHARMACOLOGY

DEVELOPMENTAL THERAPEUTICS PROGRAM

NATIONAL CANCER INSTITUTE

July 1, 1976 - September 30, 1977

This laboratory is engaged in studies which approach the cancer chemotherapy problem at the level of molecular and cellular biology. The emphasis currently is on anti-tumor agents which directly attack DNA, particularly alkylating agents, nitrosoureas, platinum complexes and ionizing radiation. These studies have been facilitated by new methods developed in this laboratory which make it possible to measure DNA damage in cells treated with pharmacologic doses of these agents.

The first objective, and the major one addressed during the current year, is to define the types of DNA damage which are produced by various agents and which can be measured using the new alkaline elution methodology. An integral part of this phase of the problem is further development of the new methodology. A significant development during the past year has involved the addition of a proteinase digestion step which subdivides DNA crosslinking into 2 categories, presumed to be DNA-protein links and DNA interstrand crosslinks. We are now able to detect the following types of DNA damage: single-strand breaks, alkali-labile sites, DNA-protein links and DNA interstrand crosslinks.

The DNA damage produced by various agents is first studied in mouse L1210 cells in culture, because of the uniformity and convenience of this cell system, and because cell survival can readily be measured. During the past year the types of DNA damage produced and the kinetics of formation and repair of DNA lesions have been examined in this sytem for the following drugs: nitrogen mustard, melphalan, BCNU, CCNU, chlorozotocin, FCNU, methylnitrosourea, cis-Pt (II) and trans-Pt(II).

Nitrogen mustard (HN2) and melphalan were found to produce DNA-protein links and DNA interstrand crosslinks, but with different kinetics of appearance and disappearance, both between the 2 drugs and between the 2 types of lesions. The results obtained with these 2 nitrogen mustards, particularly the marked kinetic differences suggest an approach which may be termed "intracellular pharmacokinetics," in which rates of appearance and disappearance of specific macromolecular lesions at drug reaction sites could be determined in various cells or tissues and attempts made to relate these phenomena with cell killing.

The results with cis-and trans-Pt(II) were of the same sort as with the nitrogen mustards, except that the trans isomer (which lacks anti-tumor activity) appears to generate solely DNA-protein links, whereas the active cis-isomer produced both types of crosslinks. Such comparisons between active and inactive modifications of anti-tumor compounds may lead to identification of these types of DNA damage which are crucial for anti-tumor activity.

The chloroethylnitrosoureas, BCNU, CCNU and chlorozotocin, gave more complex results, because in addition to the 2 types of crosslinks, single-strand breaks also were formed. These agents were found to differ from each other in the rates of crosslink repair.

The second objective is to determine whether there are differences in drug-induced DNA damage and repair between different cell types, particularly between mouse and human cells and between normal and neoplastic or transformed cells. The importance of these questions is emphasized by the known differences in DNA repair mechanisms between mouse and human cells, and by the obvious desirability of studies to define differential toxicity between normal and neoplastic human cells.

Normal human embryo cells and a virus-transformed derivative line have been used in this laboratory, and cell cloning techniques were developed to permit comparative studies of survival of proliferative ability. In this system, differential killing of the transformed cell type was noted with several chloroethylnitrosoureas but not with ionizing radiation.

We have compared the effects of BCNU on the normal and virus-transformed human embryo cells, using both alkaline elution and alkaline sedimentation techniques to observe the formation and disappearance of DNA single-strand breaks. The results have led to a conjecture, which is being further tested, that the 2 types of cells differ in the ratio of actual DNA breaks to alkali-labile sites formed following drug treatment. Such a difference would indicate a difference in the activity of DNA repair steps.

This system was also used to study BCNU plus X-ray combinations, since BCNU had previously been noted to inhibit the repair of X-ray-induced single-strand breaks. The results showed that the inhibition of this repair by BCNU is much more complete in the virus-transformed cell than in the normal cells. Because of the clinical implications of this finding, similar experiments were performed on human Burkitt lymphoma cells. The results were intermediate between the normal and transformed human embryo cells. Further studies are needed on the timing of X-ray in relation to BCNU treatment and to compare different lymphoma lines.

Three human colon carcinoma lines with different sensitivities to MeCCNU were compared in regard to the formation and disappearance of DNA crosslinks following treatment with this drug. Studies were performed after in vivo treatment of the tumors in nude mice and in vitro in the corresponding cell lines. Differences between tumor lines were noted which may be related to the difference in drug sensitivity. However, the findings in vivo and in vitro failed to provide a consonant picture, suggesting that in vitro culture may have altered the cells. We plan to further pursue this work in mouse ascites tumor lines with known differences in drug sensitivities, and in drug resistant cell culture lines. This will provide much more controlled systems for studies which will provide a better foundation for studies of human material.

Subsequent objectives of this line of investigation will be to correlate DNA damage with cell lethality and to develop a predictive test for chemotherapeutic effectiveness of particular drugs against tumors in individual patients.

In pursuing the molecular details of DNA damage produced by drugs, such as alkylating agents, it is of interest to identify the proteins which become linked to DNA and to determine whether this differs depending on the drug. Procedures were developed to isolate proteins linked to DNA by nitrogen mustard. The linked proteins were released at low pH at rates similar to the release of bound nitrogen mustard, indicating that the proteins are linked to purines at the expected sites of alkylation. The linked proteins were found to be largely non-histones, although gel electrophoresis disclosed a band which may be one of the histones. In order to characterize the bound proteins, peptide fingerprinting is being performed . Different drugs known to bind protein to DNA will be compared to see whether there are differences in the proteins which become bound.

In addition to cell lethality, DNA damage also produces mutagenesis. The relative extents of the lethal and mutagenic actions of several drugs were determined in order to relate them to the DNA damage produced. The objectives are 2-fold: (1) to associate particular types of DNA lesions with cytolethality *versus* mutagenesis, and (2) to provide comparative information on the potential carcinogenic risk by various anti-tumor drugs. A sensitive mutagenesis assay was set up based on the development of resistance to thioguanine in Chinese hamster cells. The ratio of mutagenicity to toxicity was found to be much higher for methylnitrosourea and streptozotocin than for BCNU, CCNU or chlorozotocin. This is consistent with the idea that DNA methylation produces mainly mutation, while DNA chloroethylation has a much greater lethal potential. A high degree of mutagensis was also noted with *cis*-Pt(II). The concomitant effects of these drugs on DNA in this cell line is under study by alkaline elution.

A by-product of this work during the past year was the discovery that room fluorescent light can be highly mutagenic and produce DNA single-strand breaks in Chinese hamster cell cultures, while producing little cell lethality. The DNA single-strand breaks were detected by alkaline elution and were subject to repair at a rate resembling the rate of repair of X-ray-induced breaks. In addition to its intrinsic interest, this finding provides a new way to produce non-lethal single-strand breaks in cells for mechanistic studies.

Although currently available methodology facilitates studies of drug-induced damage to DNA, an important aspect of chemotherapeutic mechanisms is likely to be the structure of the chromatin in which the DNA resides. In order to explore opportunities for relevant investigations in this area, methods were set up and improved for the study of the nucleosomal structure of chromatin. Current work centers on the determination of inter-histone crosslinking patterns by gel electrophoresis and peptide fingerprinting techniques. These studies are directed towards possible characterization of chromatin differences in normal, neoplastic and drug-treated cells.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF
HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06112-05 LMPH

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Effects of Chemotherapeutic Agents on DNA

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	K. W. Kohn	Chief, Lab. of Mol. Pharmacol.	LMP NCI
	L. C. Erickson	Staff Fellow	LMP NCI
	W. E. Ross	Clinical Associate	LMP NCI
	C. B. Thomas	Staff Associate	LMP NCI
	L. A. Zwelling	Clinical Associate	CM NCI
	M. O. Bradley	Sr. Staff Fellow	LMP NCI
	R. Osieka	Guest Worker	LCHPH NCI
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COOPERATING UNITS (if any)
Clinical Pharmacology, Medicine Branch, NCI
Tumor Biology Section, Laboratory of Chemical Pharmacology, NCI

LAB/BRANCH
Laboratory of Molecular Pharmacology

SECTION

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS: 5.5	PROFESSIONAL: 3.8	OTHER: 1.7

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project's objectives are to characterize the formation and repair of DNA damage after treatment of mammalian cells with anti-tumor agents. In addition to the use of standard methods, such as alkaline sedimentation, a major part of this work involves the development and application of a new set of techniques known as DNA alkaline elution analysis. These techniques now provide means to estimate the following types of DNA damage at pharmacologic dosage levels: single-strand breaks, alkali-labile sites, DNA-protein links, and DNA interstrand crosslinks. The drugs studied during the past year include nitrogen mustard (HN2), melphalan, cis-Pt(II), and nitrosoureas--including BCNU, CCNU, MeCCNU, chlorozotocin, and methylnitrosourea. The kinetics of formation and repair of various types of DNA damage have been examined in mouse L1210 cells, and in human embryo cells--both normal and viral-transformed, Burkitt lmphoma cells, and in human colon carcinoma lines--both in nude mice and in cell culture. These studies are aimed at elucidating the way in which DNA damage may lead to cell death, particularly differential cytotoxicity, and providing means by which predictive sensitivity tests might be developed for clinical applications.

Project Descritpion:

Objectives:

(1) To determine the effects of chemotherapeutic agents on DNA structure and function in cells.

(2) To determine the effects of drugs on DNA replication and repair mechanisms.

(3) To relate the above effects with lethality in various cell types.

Methods Employed:

(1) Cell culture of various rodent and human cell lines--treatment with chemical agents *in vitro*; radioactive labeling of macromolecules.

(2) DNA damage analysis by alkaline elution methods developed in this laboratory.

(3) X-irradiation of cell cultures.

(4) Preparative and analytical ultracentrifugation.

(5) Measurement of DNA single-strand length distributions by alkaline sucrose gradient sedimentation.

(6) Gel electrophoresis and peptide fingerprint characterization of nuclear proteins.

(7) Growth and *in vivo* treatment of human tumor xenografts in nude mice X-irradiation of cell cultures.

(8) Hypaque-Ficoll gradient separation of viable cells from tumor homogenates.

Major Findings:

1) Characterization of DNA Lesions that are Measurable by Alkaline Elution

Several major classes of anti-cancer agents are known to attack DNA, and this action may be a means by which therapeutic effects are produced. Studies of effects on DNA in treated cells have recently been facilitated by new methodology developed in this laboratory based on measurements of the rates of elution of DNA single-strands from alkaline cell lysates trapped on filters.

Previous work had shown how alkaline elution measurements can be used to quantitate DNA single-strand breakage and DNA crosslinking. Single-strand breaks are revealed by an increase in DNA elution rate, while crosslinking is shown by an inhibition of the ability of X-ray-induced single-strand breaks to increase elution rate. Studies during the past year have enlarged the list of measurable lesions to 4 types which can be at least partially resolved. Increases in elution rates were found to be of 2 kinetic types: an essentially first-order type and an accelerated type in which the rate increases with time. We attribute the former type to DNA breaks existing in cells, and the latter type to alkali-sensitive DNA alterations which are only converted to breaks during the elution procedure. The latter type was clearly seen with methylating agents such as methylnitrosourea and methylmethanesulfonate. Work described under section 5 however suggests that these lesions may have importance in developing an understanding of the differential toxicity of BCNU.

The crosslinking measurement was also resolved into 2 components, based on the ability of the crosslinking effect to be reversed by proteolytic digestion of the cell lysate. The proteinase-sensitive component is thought to represent DNA-protein links, while the proteinase-resistant component is thought to represent interstrand crosslinks. Both types of crosslinks were produced by nitrogen mustards, nitrosoureas, and platinum complexes.

2) DNA Lesions in L1210 Cells Due to Nitrosoureas

We had previously obtained evidence that BCNU and other chloroethylnitrosoureas produce interstrand crosslinks in purified DNA. We had also found in alkaline elution experiments that BCNU produces crosslinks in L1210 cells and additionally that it produces single-strand breaks. The formation of crosslinks was in both studies found to be a delayed reaction which proceeded for several hours after removal of drug. The delayed kinetics was attributed to the time required for completion of the second reaction in a crosslinking sequence.

We have now extended the alkaline elution studies in L1210 cells to other nitrosoureas, and we have applied the proteinase digestion method to distinguish DNA-protein and DNA interstrand crosslinks.

In addition to BCNU, delayed crosslinking was observed with CCNU and chlorozotocin. With all 3 drugs, crosslinking was initially of the DNA-protein type, and DNA interstrand crosslinks accumulated after a delay of a few hours. CCNU and chlorozotocin however differed from BCNU in the repair of crosslinks. With the former 2 drugs, crosslink removal was evident 24 hrs after treatment, and was more prominent for interstrand crosslinks than for DNA-protein links. With BCNU, on the other hand, neither type of crosslink was removed.

In the case of FCNU (the fluoro analogue of CCNU) only an initial crosslinking of the DNA-protein type was seen, and there was no post-treatment increase in crosslinking, and no interstrand crosslinking. This agrees with previous findings that FCNU does not produce interstrand crosslinking in purified DNA, and is consistent with the expected reduced alkylating activity of the fluoro substituent.

3) The Kinetics of Formation and Disappearance of DNA Crosslinks in L1210 Cells Treated with HN2 and Melphalan

Marked differences were observed between HN2 and melphalan in regard to the formation and disappearance of DNA crosslinks in cultured L1210 cells. Cells were treated with drug for 30 min, and then incubated in the absence of drug for various periods of time prior to assay by alkaline elution. The kinetics following HN2 was dominated by an exponential disappearance of crosslinks with a half-time of 7 hrs., beginning after a 2-hr delay following removal of drug. Melphalan, on the other hand, exhibited a marked rise in crosslinking for about 4 hours after removal of drug, and this was followed by a much slower rate of crosslink removal than was the case with HN2.

These events however are different for DNA-protein links as opposed to interstrand crosslinks. Work is now in progress to determine the kinetics for the 2 individual types of crosslinks.

The objective will be to determine whether a molecular parameter can be identified which correlates with cell survival.

4) The Kinetics of Formation and Disappearance of DNA Crosslinks in L1210 Cells Treated with *Cis*-and *Trans*-$Pt(II)(NH_3)_2Cl_2$

It had been reported by others that platinum complexes produce DNA interstrand crosslinks, both in purified systems and in cells. The previous studies in cells, however, required the use of supra-toxic drug concentrations because of the relatively low sensitivity of the methods used. Although the *cis*-Pt(II) isomer is much more cytotoxic and active as an antitumor agent than is the *trans* isomer, the relative extents of DNA crosslinking by the 2 complexes has been in doubt. We therefore attacked the problem using the alkaline elution technique.

As expected, L1210 cells were much more sensitive (by survival of colony formation) to *cis* than to *trans*-Pt. Both isomers however produced measurable crosslinking at sub-toxic doses, but the kinetics were markedly different. The extents of crosslinking following 30-min exposures to *cis*-Pt were initially low and then exhibited a large rise for up to 12 hrs. after removal of drug, followed by a phase of gradual loss of crosslinking. The *trans* isomer, however, showed maximum crosslinking immediately after removal of drug, followed by a steady loss of crosslinks.

Because of the large difference in cytotoxicity of the 2 platinum isomers and the high extents of crosslinking exhibited by both agents, we expect this system to provide an excellent test of the question of whether crosslinking events correlate with cytotoxicities. This is being approached by comparative experiments using equitoxic doses and by assays to separate DNA-protein and DNA interstrand crosslinks.

5) Studies of DNA Damage Produced by BCNU in Normal (WI-38) and Transformed (VA-13) Human Embryo Cells

These studies were undertaken in order to determine whether the origin of differential cytotoxicity could be investigated in human cell of normal and transformed phenotype. The 2 cell lines exhibited differential sensitivity to the drug, with the transformed line being more readily killed. The major findings of the past year concern the production of single-strand breaks by BCNU. Single-strand breaks were noted both by alkaline sucrose gradient sedimentation and by alkaline elution. The break frequencies however appeared to be much larger when estimated by alkaline sedimentation than when estimated by alkaline elution. Furthermore, alkaline sedimentation indicated more breaks in the normal line than in the transformed line, while alkaline elution indicated the reverse. This contrasted with a variety of previous studies in which results by the 2 methods were in excellent agreement. Our working hypothesis, which is currently under test, is that alkaline sedimentation--which is conducted at a higher pH than is alkaline elution--measures both actual breaks and alkali-labile sites, while alkaline elution measures mainly actual breaks. This would suggest that the ratio of actual breaks to alkali-labile sites may be greater in the transformed than in the normal cell type. Such a difference could have important implications in regard to differences in the DNA repair sequences in the 2 cell types.

6) Differential Inhibition by BCNU of Repair of X-ray-Induced Single-Strand Breaks in WI-38 and VA-13 Cells

We have previously reported a differential interaction of BCNU and X-ray in WI-38 versus VA-13 cells, in regard to the formation and/or repair of DNA single-strand breaks.

The previous results, which were obtained using the alkaline sedimentation method, were extended using alkaline elution. Further experiments also indicated that the effect of BCNU on VA-13 cells is to halt the rejoining of X-ray-induced single-strand breaks, and that WI-38 cells are affected to a lesser degree.

Similar studies were carried out in human Burkitt lymphoma cells using the alkaline elution technique. The magnitude of the interaction between BCNU and X-ray was found to be intermediate between that observed with WI-38 and VA-13 cells.

7) DNA Crosslinking by MeCCNU in Human Colon Carcinoma Lines in Culture and in Nude Mice

Human colon carcinoma lines BE and HT have been established both as cell culture lines and as transplantable tumors in nude mice. In both systems, line BE was found to be more sensitive than line HT to MeCCNU. Our objective was to determine whether these lines exhibited response differences to MeCCNU that could be observed in terms of DNA crosslinking.

In order to carry out alkaline elution assays on the solid tumors from nude mice, a method was developed to prepare cell suspensions suitable for alkaline elution assay. Although the tumors contained a variable fraction of necrotic cells, the DNA contribution from such cells could be discounted by ignoring the initial component of rapid elution which was present to a variable degree in these samples. In this way, reproducible effects of MeCCNU on DNA in the form of crosslinking were observed after in vivo treatment.

Treatment of the cells in culture failed to show significant differences in over-all crosslink formation. In both cell types, crosslinking was observed to increase during 6 hrs. of incubation after drug removal. Between 6 and 24 hrs. after treatment, however, crosslink removal was significantly greater in the resistant HT line than in the sensitive BE line.

After in vivo treatment however a different type of result was obtained: the resistant line developed less crosslinking than the sensitive line. Apparently these cell lines behave differently upon in vitro than in vivo treatment to MeCCNU. The source of this difference remains to be determined.

8) Assistance in Establishing Alkaline Elution Technique in Other Laboratories

With an expanded interest in the alkaline elution technique, a substantial activity in our laboratory during the past year was to provide training and assistance in establishing the technique in other intramural and extramural laboratories.

Proposed Course:

1) Develop a physical model to permit more precise description of DNA elution kinetics.

2) Further characterize the effects ascribed to DNA-protein and DNA interstrand crosslinking by quantitative studies based on physical models.

3) Develop an improved assay for alkali-labile sites, based on the rate of development of DNA elutability by incubation at high pH. Use it to test the conjecture (section 5 above) that WI-38 and VA-13 cells differ in the ratio of alkali-labile to single-strand breaks following treatment with BCNU.

4) Determine the rates of formation and disappearnace of DNA-protein and DNA interstrand crosslinks using the proteinase-alkaline elution technique. Effects of HN2, melphalan, BCNU, cis-Pt and related compounds will be examined in L1210, WI-38 and VA-13 cells.

5) Select L1210 clones specifically resistant to HN2, BCNU or cis-Pt, and compare them in regard to the formation and repair of DNA lesions.

6) Develop an in vivo-in vitro procedure to determine relative responsiveness of various mouse L1210 ascites tumor lines. Attempt to correlate in vivo responsiveness of resistant tumor lines with in vitro effects on DNA measured

by alkaline elution. Drugs of primary interest will be melphalan, BCNU and cis-Pt.

7) Determine the responsiveness of various Burkitt lymphoma lines to in vitro treatment with alkylating agents, nitrosoureas and platinum complexes, using alkaline elution procedures to measure DNA damage and repair.

Publications:

1. Fornace, A.J., Jr., and Kohn, K.W.: DNA-Protein Cross-linking by Ultraviolet Radiation in Normal Human and Xeroderma Pigmentosum Fibroblasts. Biochim. Biophys. Acta 435, 95-103, 1976.

2. Kohn, K.W., Erickson, L.C., Ewig, R.A.G., and Friedman, C.A.: Fractionation of DNA from Mammalian Cells by Alkaline Elution. Biochemistry 15, 4629-4637, 1976.

3. Iqbal, Z.M., Kohn, K.W., Ewig, R.A.G., and Fornace, A.J., Jr.: Single-Strand Scission and Repair of DNA in Mammalian Cells by Bleomycin. Cancer Res. 36, 3834-3838, 1976.

4. Kohn, K.W., and Ewig, R.A.G.: Effect of pH on the Bleomycin-induced DNA Single-Strand Scission in L1210 Cells and the Relation to Cell Survival. Cancer Res. 36, 3838-3841, 1976.

5. Kohn, K.W.: Interstrand Cross-linking of DNA by 1,3-Bis(2-chloroethyl)-1-nitrosourea and Other 1-(2-haloethyl)-1-nitrosoureas. Cancer Res. 37, 1450-1454, 1977.

6. Ewig, R.A.G., and Kohn, K.W.: DNA Damage and Repair in Mouse Leukemia L1210 Cells Treated with Nitrogen Mustard (HN2), 1,3-Bis(2-chloroethyl)-1-nitrosourea and Other Nitrosoureas, Cancer Res. 37, 2114-2122, 1977.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 06132-01 LMPH

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Biological Effects of Chemotherapeutic Agents on Cultured Human and Rodent Cells

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	M. O. Bradley	Sr. Staff Fellow	LMP NCI
	L. C. Erickson	Staff Fellow	LMP NCI
Other:	K. W. Kohn	Chief, Lab. of Mol. Pharmacol.	LMP NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Molecular Pharmacology

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INSTITUTE AND LOCATION
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TOTAL MANYEARS: 2.5	PROFESSIONAL: 1.2	OTHER: 1.3

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☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Our work is in two major areas. The first goal is to understand and then enhance differential toxicity between transformed and normal cells. We are working with lines of normal and transformed human cells in culture and have shown that a metabolic inhibitor will protect normal but not transformed cells from killing by a cycle specific agent leading to a greatly enhanced therapeutic ratio. We have shown that some of the nitrosoureas selectively kill transformed human cells more than normal and we are attempting to elucidate the mechanisms involved. We have done preliminary work suggesting that the differential toxicity of BCNU for transformed cells can be enhanced by X-ray. Our second goal has been to describe the relationship between toxicity and mutagenicity for a number of classes of anti-tumor agents in cultured mammalian cells. We have correlated these biological data with DNA single-strand breaks and crosslinks. Our goal is to find DNA lesions produced by certain agents that retain toxicity and anti-tumor activity but lack mutagenic and carcinogenic activity. As a ramification of this work we have shown that fluorescent light is highly mutagenic to mammalian cells and that it causes DNA single-strand breaks, some types of which may result in toxicity and mutagenicity and others of which do not.

Project Description

Objectives:

One objective is to pursue the differential toxicity produced by certain nitrosoureas in our normal/transformed human cultured cells. We will first determine the quantitative degree of differential toxicity produced by various agents and then attempt to relate these findings to drug structure and types of DNA damage. These studies should lead to an increased understanding of the basis of differential toxicity.

Using the same human normal/transformed cell system we will examine with alkaline elution what changes occur in the DNA of normal cells as they move from an arrested Go state into the cell cycle. These experiments will test the hypothesis that DNA single strand breaks are necessary in order to change inactive chromatin so that DNA synthesis can proceed. Such information will help us understand mechanisms of growth control and may give us clues again as to how to enhance differential toxicity.

We have preliminary data showing that X-ray in combination with BCNU can enhance by 10-100 fold the killing of transformed as opposed to normal cells. We will attempt to reconfirm these experiments, to extend them to other agents, and to suggest optimum scheduling.

The nitrosoureas are much more active against experimental mouse tumors than against _in situ_ human tumors. We will examine a cultured mouse normal-transformed cell system to determine whether nitrosoureas cause a greater differential toxicity in mouse than in human cells. The answer to this question will help decide how important differential neoplastic cellular toxicity is for anti-tumor response.

We will continue to analyze the relationship between toxicity, mutagenicity, and DNA damage for a number of anti-tumor agents. Our goal is to determine which drugs and which types of DNA lesions provide the best compromise between anti-tumor activity and carcinogenicity so that long term therapy can be made more efficacious and the incidence of second malignancies reduced. We will continue calibrating this system with a number of well known carcinogens such as X-ray, ultraviolet light, and chemicals so that a rational basis for drug compairson can be achieved. Mutagenicity and toxicity will be correlated with DNA strand breaks and crosslinks in the same cell system and at low drug concentrations so that the biological consequences of particular DNA alterations can be better understood.

Our work with fluorescent light will include further studies on its mechanism of mutagenesis and toxicity, its carcinogenic potential, its effect on DNA, and its relationship to chemical damage to DNA. We hope that this work, while interesting in its own right, will also help us to further evaluate the relationship between particular types of DNA damage and their consequent biological effects. The lesions caused by these light sources may be similar to those produced by certain chemical agents that produce a spectrum of biological effects such as toxicity, mutagenicity carcinogenicity, and anti-tumor activity. Our hope is that further understanding of lesions produced by both light and chemicals may lead to the rational design of drugs that retain desirable properties such as anti-tumor activity but lack others such as mutagenicity and carcinogenicity.

Methods:

(1) Cloning and monolayer culture of WI-38, VA-13, CHO, and CHL cells.

(2) Mutation assays using the V-79 Chinese Hamster Lung cell and 6-thioguanine resistance.

(3) Measurements of DNA repair using alkaline elution and alkaline sucrose gradients.

(4) Inverted phase photomicroscopy

(5) Incorporation studies of radioactive drugs and DNA, RNA, and protein precursors.

(6) Pulse-chase and approach to equilibrium measurements of protein degradation in normal and transformed human cells.

Major Findings:

(1) The normal human fibroblasts, WI-38, can be protected from the cytotoxicity of hydroxyurea if it is held in G1 or Go during drug treatment. This protection was activated by both density-dependent inhibition of growth and by puromycin aminonucleoside. In contrast, SV40 transformed WI-38 (VA-13) are killed by the same protocols because they lack the normal cell cycle controls. These results suggest that a search for agents that selectively and reversibly inhibit normal cycling human cells might lead to an enhancement of differential tumor toxicity.

(2) In WI-38, a normal human fibroblast, the rates of degradation of short lived and long lived proteins are identical whether the cultures are growing exponentially or are density-inhibited. Replacement of the growth medium with fresh medium does not alter these rates. In VA-13, an SV40 transformed derivative of WI-38, the rates of protein degradation are also indepenent of growth rate and fresh medium. However, in both WI-38 and VA-13 the rate of long lived protein degradation increases as the serum concentration is reduced below 5%. After complete serum withdrawal, the rate increases by 60 to 100% in both cell types. Withdrawal of arginine and phenylalanine triples the rate of long lived protein degradation, while addition of 10% dialyzed serum to this amino acid-deficient medium reduces the effect to twice that of the controls. Incubation of both types of cells in phosphate-buffered saline also increases protein degradation. This effect is reduced by glucose, albumin, and dialyzed serum. Therefore, the rate of protein degradation is indepeneent of growth rate in normal and transformed human cells. However, the rate of degradation is closely coupled to certain medium alterations.

(3) Fluorescent light is both toxic and mutagenic to cultured hamster cells. The effect appears to be due to a direct interaction of light with the cells themselves and or to reactions with the culture medium. We conclude that exposure to fluorescent light should be limited during virtually any study with cultured cells. It is important that all exposure to light in our work was through the plastic tops of the culture vessels. Plastic absorbs more than 99% of any ultraviolet wavelengths less than 295 nm, so that the active wavelengths are presumably longer than 295 nm. Exposure to excessive fluorescent light might produce artifacts in studies of in vitro toxicity, mutation, transformation, chromosome variation, aging, effects of radiation, drug interactions and biochemistry. Whether or not our findings with V-79 Chinese hamster cells will extend to human and other mammalian cells remains to be determined. If human cells are also caused to mutate by fluorescent light, then the advisability of limiting human exposure to fluorescent light should be considered.

(4) Fluorescent light induces a great number of single-strand breaks in the DNA of cultured Chinese hamster cells. The breaks are induced at 1°C and are not the result of endonuclytic incision. The lesions producing the breaks are apparently not alkaline sensitive. When the cells are incubated at 37°C, the breaks are rapidly rejoined in a manner similar to the repair of X-ray damage. At least two lesions are responsible for the observed effect. One lesion has the ability to break DNA in alkali but is neither toxic nor mutagenic. This lesion is produced by light of wavelength greater than approximately 350 nm. The other lesion(s) also produce strand-breaks but may be responsible for mutagenicity and/or toxicity. These lesion(s) are produced by light of wavelength less than 350 nm.

(5) Nitrosoureas are more cytotoxic for transformed than normal human cells in culture. CNU and CCNU provide a greater selective toxicity than does BCNU or MNU. The selective toxicity of BCNU for transformed cells may be synergistically enhanced by X-ray.

(6) Quantitative comparisons of the mutagenicity and toxicity of BCNU (NSC 409962), CCNU (NSC 79037), CHLZ (NSC 178248), CNU (NSC 47547), FCNU (NSC 87974), MNU (NSC 23909), and STRZ (NSC 85998) were made in V-79 Chinese hamster lung cells. Mutant frequency after drug exposure and appropriate expression time was assayed as the proportion of cells resistant to 6-thioguanine, a marker for inactive HGPRT. Toxicity was measured as the loss of cloning efficiency. The results show that at 20 μM all the drugs cause an approximately 10-fold increase in mutant frequency over the controls. However, at a concentration of 40 μM, BCNU is almost 4 times more mutagenic than MNU. When mutagenicity is compared to toxicity the results show that STRZ and MNU are highly mutagenic at low toxicities whereas the other nitrosoureas tested were highly mutagenic only at high toxicities. A clonal line of V-79 cells was selected for resistance to BCNU. The mugatenicity of BCNU in the resistant line is higher at the same toxicity when compared to the wild type line. The results show that the chloro-and fluoro-ethyl nitrosoureas generate significant toxicity before many mutagenic lesions accumulate, whereas the methyl-nitrosoureas do not generate toxicity without concomitant mutagenicity.

(7) Cis-diamino-dichloro-platinum II is highly mutagenic to V-79 Chinese hamster cells whereas the trans analog is not mutagenic even at very high toxicities. This finding implies that the mechanisms of cellular toxicity are different for the 2 analogs.

Proposed Course:

(1) To perform cloning experiments with normal and transformed human cells to determine which nitrosoureas provide the most differential toxicity for transformed cells. We will attempt to determine which type of drug structure, provides the greatest degree of selective toxicity. The drugs studied will be BCNU, MNU, CCNU, CNU, FCNU and chlorozotocin.

(2) To continue the combination of X-ray with BCNU, CNU, and chlorozotocin to see if these combinations will enhance selective toxicity.

(3) To perform experiments similar to (1) above with normal and transformed mouse cell lines.

(4) To continue studies of toxicity and mutagenicity with selected anti-tumor drugs of interest such as cross-linking agents, intercalators, anti-metabolites, and platinum complexes.

(5) To determine whether anti-oxidants such as ascorbic acid, vitamin E, BHT, etc. and the enzymes glutathione peroxidase, and superoxide dismutase can prevent the effects of fluorescent light on cells. To determine whether DNA strand-breaks by fluorescent light occur directly or require an intact cell. To determine a partial action spectrum for the fluorescent light effects.

(6) To continue correlations of the biological effects of mutation and toxicity with the types of DNA damage produced as measured by elution and sedimentation methods.

Publications:

1. Bradley, M.O., Erickson, L.C., and Kohn, K.W.: Normal DNA Strand Rejoining and Absence of DNA Crosslinking in Progeroid and Aging Human Cells. Mutation Res. 37, 279-292, 1976.

2. Bradley, M.O., and Sharkey, N.A.: Mutagenicity and Toxicity of Visible Fluorescent Light to Cultured Mammalian Cells. Nature 266, 724-726, 1977.

3. Bradley, M.O.: Regulation of Protein Degradation in Normal and Transformed Human Cells. J. Biol. Chem., in press, 1977.

4. Bradley, M.O., Kohn, K.W., Sharkey, N.A., and Ewig, R.A.G.: Selective Protection of Normal but not Transformed Human Cells from Hydroxyurea Killing by Aminonucleoside of Puromycin. Cancer Res., in press, 1977.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 CM 06140-01-LMPH

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)
Protein Interactions in Chromosomes

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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	C. B. Thomas	Staff Associate	LMP NCI

COOPERATING UNITS (if any)
None

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Protein interactions in chromosomes are being studied with crosslinking reagents and chemotherapeutic agents. A method has been developed for isolating and characterizing proteins crosslinked to DNA. Nitrogen mustard was found to crosslink certain nonhistone proteins, and possibly one histone to DNA.

Histone-histone interactions are also being studied with crosslinking reagents. Water soluble carbodiimide crosslinks histones in chromosomes to one another, the major product being a H3-H4 dimer. Other products are being characterized to obtain some insight into histone-histone interactions.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives:

The overall objectives are to help elucidate the role of chromosomal proteins in the structure and function of chromosomes, and to apply this knowledge to understanding and treating cancer. Specific objectives are to:

(1) Determine the effect of chemotherapeutic agents on the DNA-protein interactions.

(2) Determine the spatial relationship of histones in chromosomes.

Methods:

(1) Crosslinking of protein to protein using bifunctional reagents.

(2) Separation of crosslinks on acrylamide gels.

(3) Efficient recovery of proteins from gels (new method developed in this laboratory).

(4) Fingerprint analysis to determine the identify of proteins in crosslinked structures.

(5) Efficient detection of H^3 and C^{14} on thin layer plates (new method developed in this laboratory).

(6) Crosslinking of protein to DNA using bifunctional chemotherapeutic agents.

(7) Isolation of proteins crosslinked to DNA by centrifuging through CsCl.

Major Findings:

1) Protein Crosslinking to DNA

A protocol was developed for isolating proteins crosslinked to DNA. Proteins crosslinked to DNA by nitrogen mustard were released from the DNA at pH 4.3 with kinetics very similar to nitrogen mustard release, indicating that the mustard-DNA link was through an altered purine in both cases. Analysis of the proteins crosslinked to DNA indicated that they are mainly nonhistone proteins.

Surprisingly, the evidence indicates that histones are very poorly crosslinked even though they account for half of chromosomal protein. One putative histone was found. If this assignment is substantiated through fingerprinting, then HN2 shows considerable selectivity in crosslinking histones.

2) Structure of the Nucleosome

Histones in chromosomes are arranged in discrete bodies called nucleosomes. Using bifunctional protein-protein crosslinking reagents, we are investigating the arrangement of the histones in the nucleosome. Previously a (histone) H3-H4 crosslink was found with carbodiimide. Using chemical and enzymatic methods, this crosslink has been localized to the central portion of each amino acid sequence. Also several new crosslinks have been identified, the major ones being between histones H3-H2a, H3-H2b and a minor one H2a-H2b. With a different reagent, succinimidyl propionate the crosslinks H4-H2a, H4-H2b, H4-H3, H3-H2b have been found. Using this data, and other data in the scientific literature, a detailed picture of histone-histone interactions in the nucleosome is developing.

Histones H2a and H2b can be removed from nucleosomes with 1.2M salt. The resulting structure still has several nucleosomal properties such as the ability to put supercoils into DNA. A comparison of the crosslinking pattern of the partial and the whole nucleosome indicates that the H2a-H2b pair binds between two H3-H4 pairs.

Significance to Biomedical Research and the Program of the Institute

The DNA protein crosslinks induced by chemotherapeutic agents may be an important aspect of their function. Therefore our understanding of this parameter for these drugs could lead to a fuller understanding of their action and be potentially useful in designing drugs of greater selectivity.

The work on nuclesome structure can lead to a greater understanding of chromosome structure and perhaps indicate differences in normal and neoplastic cells that may be exploited for chemotherapeutic purposes.

Proposed Course:

1) To compare the proteins crosslinked to DNA by other chemotherapeutic agents besides nitrogen mustard.

2) To look for differences in nucleosome structure between active and inactive regions of chromosomes and between normal and neoplastic cells.

Publications:

None

ANNUAL REPORT OF THE LABORATORY OF TOXICOLOGY

DEVELOPMENTAL THERAPEUTICS PROGRAM

NATIONAL CANCER INSTITUTE

July 1, 1976 to September 30, 1977

Introduction

The Laboratory of Toxicology continues to try to honor its dual responsibilities for conducting basic research and for the supervision of large animal toxicologic studies of antineoplastic agents prior to their introduction into man. This important latter role had actually been carried out in the past by a rather diffuse contract mechanism, with the Laboratory of Toxicology retaining direct responsibility to supervise these studies, summarize the information, and aid in the submission of an INDA to the Food and Drug Administration before a Phase I Clinical Trial is initiated. In February of 1974, the Division awarded a Prime Contract, wherein the responsibilities for these studies are shared by the Prime Contractor (Battelle Memorial Institute) and this Laboratory. Undoubtedly this Prime Contract made available more time for the inhouse staff to properly pursue some important aspects of research into toxicological mechanisms of action of antineoplastic agents.

For the next fiscal year the laboratory is committed to three major goals: 1) to attempt to recruit high quality replacements for the individuals who are leaving LT; 2) to continue the improvement of the inhouse basic research; and 3) to cooperate with the Prime Contractor in maintaining the excellent, technical momentum achieved during the first three contract years. It is felt that with the continuing limitations of staff, it would be unrealistic to project additional goals. On the other hand, if staff conditions are significantly improved it is the strong desire of the Laboratory to define and support research in the area of delineating mechanisms of toxicity of anticancer agents with the hope of proposing remedies for these toxic actions.

It is going to be extremely difficult to achieve the first goal with the personnel support the LT has had the past 5 years. Since 1970 the budget for LT has increased about 5-fold. While the actual number of committed slots at the time of the site visit was the same as 5 years ago, there were times during this interval when as many as 20% of the original slots were denied for various reasons. Hence, in five years we have achieved parity which some regard as growth! One problem from such restricted growth is that fresh viewpoints are impossible. This is because creativity is procedured and channeled to death in people long in government service. But further, even if some promising individuals had been found over the past 5 years, there has been insufficient technical and clerical help to properly support creative and productive senior staff members. Outside management consultants have suggested we have one half the staff necessary to accomplish our mission.

It is our belief that without growth, there can be only stagnation. In a desperate effort to infuse some "new blood" into this stagnant situation, we have recently entered into a new area for us, i.e., recruitment of foreign fellows via mechanisms which do not count against personnel ceilings. Of course, such individuals are not expected to help with program activities of the Laboratory, but rather it is hoped that their presence will help in the basic research areas.

For the fourth year in a row, some senior investigators have sustained superior research efforts, only by heroic personal efforts. It was predicted in the annual report of three years ago, that it was "unfair and unhealthy to expect this to continue". In all honesty it must be admitted that there is evidence of further attenuation of these heroic personal efforts. The management cry to "establish new priorities" but "within present resources" carries with it grave risks. The risk that you may terminate a highly worthwhile project because the potential is not currently recognized. Setting priorities in biomedical science simply has not achieved the fine state of art found in engineering and other technological areas.

In contemporary biomedical science, setting priorities is guess work and no matter how much information you put into a "guess", you are going to be wrong a high percentage of the time. While we have emphasized the positive aspects of the Prime Contract, it must be recognized that some features of this effort have been unpredictable and represented a greater time burden for some individuals than had been anticipated. For example, it must be pointed out that the Prime Contract takes a different kind of effort because of its "incentive" features. In other words, because the contractor is receiving a portion of his fee from this mechanism, extensive documentation is required to justify the award or denial of fee. It has been very difficult to structure the roles of the limited staff of the Laboratory in this award-fee situation. It has been recommended that there be no over-lapping roles between the persons requesting the work and the individuals recommending the award of funds. Thus, three categories of personnel are required for proper operation of the Prime Contract: 1) compound/task officers; 2) contract monitors; and 3), evaluators. It became obvious two years ago, that with the Laboratory down to five senior staff slots, the requirements that the three roles not overlap was impossible to meet. Therefore, we were obliged to functionally delete two contract monitors. Another position, that of evaluator, should be at the professional level but was accomplished by a technician for most of this past year; this technician has since resigned. Thus, we are faced with the loss of a contract monitoring position which is going to be difficult to fill because it will take a year to train a replacement.

A serious word of caution must be expressed regarding operation of the Prime Contract and that word is complacency. Because the initial procurement was well-organized and because the workscope was well-written and because the over-all performance of the contractor has been so good, this is no time to relax our monitoring job, particularly in the face of major Program changes especially regarding the number of drugs handled and degree of focus on certain antineoplastic agents.

We continue to insist, as we have in the past annual reports, that the supervision of the contractors' research activities be done by members of the scientific staff who have considerable first hand knowledge about the research problems involved with the use of our often uniquely toxic antineoplastic agents. If the internal staff can not maintain and grow in this kind of knowledge, then some important observations made by contractors can not be properly assessed.

Is there an alternative to these problems that are due to poor staffing? Of course there is the possibility of allowing LT to revert to its original status of 10 years ago with a staff of 3 with no role other than contract supervision. Of course, such monitors would be out of the rapidly advancing mainstream of toxicologic research within a few years. If one does not need higher calibre scientific direction, there are a number of mediocre individuals who would be willing to try to do this work to satisfy regulatory requirements.

II. Internal Research Programs

The independent and joint projects of the senior staff members are primarily directed toward attempting to understand the actions of antineoplastic agents, especially those actions which manifest themselves as some form of toxicity. It is only through an understanding of such actions that we will be able to advise clinicians intelligently as to what toxicity to expect, and when toxicity does occur, what steps may be taken to reverse it.

The practice of modern toxicology is really impossible to separate from pharmacology. Obviously, the properties of drugs and membranes which bring a drug to a site of pharmacologic action, can also induce toxic responses. Our general approach is quite broad because we study drugs through a series of complex processes that end in a potential lesion. Thus, we are interested in the drug during the exposure phase (bioavailability), the pharmacokinetic phase (absorption, distribution, metabolism and excretion), the pharmacodynamic phase (therapeutic) and finally, the toxicodynamic phases.

As far as the science of toxicology is concerned, those of us who work with antineoplastic agents recognize that we have an extra obligation to our colleagues working with other classes of xenobiotics. This is because oncolytic agents include some of the most toxic substances to which man is exposed. Also, while the over-all ability of animals to predict for human toxicity is poor, this is largely the result of the inadequacies of the human toxicologic data. For example, those working with pesticides must usually settle for the poorly quantified occupational exposures, as indices of human toxicity. Since we obtain both animal and human toxicity information, on a qualitative and quantitative basis, we are in a better position to assess the animal to human correlations of such data. Animal tests of our drugs have been successful, as is attested to by the relatively small number of serious toxic reactions which were not anticipated as a result of animal studies. Hence, we have regarded our scientific role as one of providing leadership in animal testing rather than "followership" of regulatory agency guidelines which would have little meaning when applied to anticancer drugs.

A. Biochemistry Section

The major mission of this Section is to characterize the biochemical basis for the toxicity of certain antineoplastic agents. A secondary role of this Section has been to develop and apply sensitive methods for the detection of enzymes, and substrates, in particular, through the use of radiometric techniques. In very complete studies of alanosine (NSC 153,353), an agent expected to enter clinical trials within a year, both areas of expertise have been utilized. Several possible metabolites have been synthesized by enzymic means and the primary urinary metabolite seems to be the α-hydroxy analog of alanosine. Since none of these metabolites is as potent as is the parent, it is likely that alanosine itself is the chemotherapeutically active species.

Similar in-depth biochemical studies are in progress for two other drugs, PALA (NSC 224,131) and an isoxazoleacetic acid analog (NSC 163,501), which presently are under preclinical development.

This Section is involved with the adriamycin-vitamin E saga, and has been able to reproduce the results of Myers, et. al., (CTR, 60: 961, 1976) in mice.

B. Comparative Pathology Section

The primary efforts in this Section have continued in the area of adriamycin (ADR) induced cardiotoxicity, the major dose-limiting toxicity in the clinic. Having established the rabbit as a reproducible model for assessing the cardiomyopathy, efforts were turned toward developing smaller animal models.

Current investigations were designed to determine if ADR would induce a significant and reproducible cardiotoxic syndrome in the rat. The majority of ADR-treated rats developed cardiomyopathy 3 to 23 weeks after the last injection. Gross evidence of congestive heart failure was also observed. The results of this study suggest that the rat model should be further developed for large scale cardiotoxicity testing.

In related studies, the Myers' hypothesis regarding the protective effects of vitamin E vs the cardiotoxicity of adriamycin was tested in the rat and rabbit. Intraperitoneal administration of vitamin E offered no protection to the toxicity of adriamycin in the rat and the rabbit in contradiction to results observed in mice. In fact, the results in these latter species not only often showed potentiations of toxicity, but provided clues regarding how the vitamin may be exerting its effects in mice. In the rat and rabbit severe peritonitis invariably occurred, thus suggesting that the sparing action of vitamin E in mice may be via protection against the chemical peritonitis caused by intraperitoneal administration of adriamycin.

This Section has published three papers in the past year establishing and utilizing the Fisher rat model for prediction of Pt-compound nephrotoxicity. It was found that both BUN values and histopathologic evaluation of renal lesions were necessary to determine the type and severity of nephrotoxicity. The model also proved useful in testing potential antidotes, e.g., furosemide diuresis, to Pt-induced toxicity.

C. Drug Interactions Section

The major thrust of·this Section is the study of "drug-drug interactions". This is particularly important since patients with neoplastic disease frequently receive a multitude of drugs which may alter the absorption, binding, distribution, excretion, metabolism or pharmacokinetics of the anticancer agent.

In a related category, drug-diet interactions also are possible and have been studied in this Section. A recent review of the nutritional status of patients with cancer (Postgrad. Med. 61: 97, 1977) pointed out that 15% have significant anorexia at the time of disease recurrence or metatasis and that with further progression, anorexia increases. This reference further states "the frequent development of protein-calorie malnutrition, vitamins and other deficiencies, and significant weight loss during the clinical course of cancer is, therefore, not surprising.... Malnourished cancer patients do not tolerate surgery, radiotherapy, and chemotherapy as well as those in a better nutritional state. The malnutrition adversely affects not only tissue function and repair, but also humoral and cellular immunocompetence, and it causes changes in drug metabolism through alterations in the activity of hepatic microsomal enzymes". Hence, methodology using antineoplastic agents, other drugs, and dietary alterations in experimental animals has been developed and applied in a number of areas.

The prinicipal activities of this Section are in the areas of drug metabolism and disposition and drug transport. Such activities are important in defining which parameters lessen drug toxicity, e.g., microsomal induction, and in understanding factors increasing toxicity. These studies have become increasingly important because of the recent evidence that drug biotransformation, leading to irreversible tissue-binding is a major parameter associated with toxicity. An increasing number of literature citations offer evidence that relatively inactive drugs can be activated *in vivo* to proximate toxicants. Over the past several years, this Section has studied hepatic, pulmonary and renal drug metabolism and now has the foundation upon which the LT can build a major five-year-program related to lung cancer.

Pulmonary neoplasms are the leading cause of cancer deaths among Americans (about 17% of all tumors in males), and, the relative incidence of lung cancer deaths among males has steadily increased since before 1940 with no hint of a plateau in sight. Only about 10% of the patients survive 3 or more years. Although surgical resection remains the mainstay of treatment (Cancer 30: 315, 1972), lung cancer is beyond the scope of surgical cure in 85-90% of all cases at the time of initial diagnosis (Cancer Chemother. Rpts. 4(3): 19, 1973), metastases to distant sites having already occurred. Progress in evolving new chemotherapy, either single agents or combinations, or combined modality therapy for malignant pulmonary tumors has been distressingly slow. To end this litany of depressing facts, current estimates suggest that up to 60-80% of human lung neoplasms are environmentally induced. In the face of this virtual epidemic of lung cancer, total lung cancer expenditures in the Division of Cancer Treatment during FY 1977 were less than 10% of expenditures, trailing funding for both colorectal and breast malignancies.

The LT has been interested in organ-specific toxicity concerning oncolytic agents in particular and xenobiotics in general and has pioneered the development of approaches for evaluating cardiotoxicity of adriamycin analogs and nephrotoxicity of Pt analogs. Thus, it seemed expedient to capitalize on this expertise by moving on to the lung as a target organ of toxicity, especially if the approach could provide a basis for improved therapeutic applications. In an initial project in this area the Drug Interactions Section and Comparative Pathology Section have been studying the dose-limiting pulmonary fibrosis associated with bleomycin administration. Experiments to prevent or reverse this lesion without reducing the oncolytic efficacy of the agent are under investigation, total lung hydroxyproline being used as a biologic marker of collagen content (and thus, fibrosis). Preliminary data indicate that this is an earlier and more sensitive index of fibrosis than other criteria examined. In a subsequent project this Section will examine bleomycin A_2 and two chemical congeners for their relative effects upon the lung.

An interesting and apparently unique property of mammalian lung is its ability to concentrate and tenaciously retain basic amine xenobiotics, particularly those with pK_a's >8.5. In some cases, tissue:plasma ratios range from 10-20:1. The affinity of compounds for the binding sites in lung varies and accordingly one drug may be released by another of higher affinity or uptake may be blocked. Thus, this Section currently is investigating the relative abilities of mouse lung and Lewis Lung carcinoma (LL) to concentrate a model basic drug, imipramine. If pulmonary tumor tissue retains the ability of normal lung to concentrate basic drugs, the basicity of potential new oncolytic agents might be utilized as a delivery system to concentrate the agent in lung, lung tumor, and extrapulmonary metastases. In experiments, designed to determine whether basic amines can displace and/or block the uptake of bleomycin from the lung and thereby reduce pneumotoxicity, an important fundamental discovery was made using the LL mouse model. It was found that neither the mouse lung, nor tumor selectively take up bleomycin and other basic drugs. This certainly raises serious questions regarding the universality of the premise that the mammalian lung concentrates basic compounds, and the use of the LL model to predict for effects in human pulmonary neoplasms. Thus in this broad research area we have the opportunity and the commitment to study the lung in relation to drug uptake and release, and toxicity (fibrosis) at the following levels of biological organization: intact animal, isolated perfused lung, lung tumors, tissue slices, isolated cell fractions, and purified drug receptors.

D. Office of the Chief

We have continued to explore the possibility of using aquatic species as models for the rapid accrual of pharmacokinetic information on antineoplastic agents and other xenobiotics. The major rationale for continuing this approach is that the rate of metabolism and of disposition of compounds administered to these animals is lower than that of mammals. To understand the mechanism and determine the kinetics of underlying pharmacokinetic phenomena, it is important to study events occurring in the initial distribution phase. In mammalian species the circulation time (blood volume/cardiac output) is typically small (i.e., less than 1 min.) compared with practicable sampling intervals. Consequently, in mammals the distribution phase for water-soluble agents occurs within the first few time points. Resolution may be improved by using a marine species with a relatively longer circulation time. Thus, we examined a model species, the dogfish shark (Squalus acanthias), which exhibits a circulation time of the order of three minutes. Since the two basic publications of last year, the model has been extended to approximately 20 antineoplastic agents with emphasis on the major routes of excretion, tissue distribution and central nervous system penetration. Plasma half-times in the dogfish shark, 50% of the drugs studied, were virtually the same as in the human; for the other drugs, no shark $t_{1/2}$ value differed from that of the human by more than 2-fold. Considering the huge phylogenic jump between shark and man, this is excellent correlation. Relative dominance of urinary vs biliary excretion of anticancer drugs in the shark appears to follow the trends reported for mammals; analysis of data for this portion of the studies are incomplete. Research of the central nervous system compartment of the shark has shown that correlations are best when 24 hr brain/plasma ratios are compared with the results of the mouse ependymoblastoma model. The three drugs with highest brain/plasma ratios in the shark, alanosine (44), ellipticine (33) and hycanthone (10), were positive in the tumor model and should be considered as candidates in human trials for CNS tumors.

The feasibility of using large volumes to administer, by the intraperitoneal route, doses of antineoplastic agents greater than therapeutically active doses, has been studied. The hypothesis is that the entire peritoneal surface is bathed in drug solution while systemic absorption and potentially lethal side-effects would be minimized. This mode of treatment could serve as adjunct therapy to surgical resection in early stages of ovarian carcinoma which are characterized by microscopic intraperitoneal metastases.

The transport of various model compounds and antineoplastic agents across the peritoneum of the rat was investigated to assess the influence of three major parameters on absorption of drug into the systemic circulation: 1) molecular weight; 2) the dissociation constant (pK_a); and 3), the lipid-water partition coefficient (K). Sprague-Dawley rats were injected intraperitoneally with radiolabeled model compounds (25) and antineoplastic agents (13) in large volumes of normal saline (50 ml). After one hour, the peritoneal fluid was removed and absorption and tissue distribution of the compounds was measured.

The 9 neutral model compounds tested ranged in molecular weight from 18 to 2 million; their peritoneal absorption varied from 69 to 2% respectively. Five compounds with variable K values were examined; for the acids, absorption increased with increasing pK_a values, while for bases the reverse was true. The absorption characteristics of 13 antineoplastic agents were determined; their absorption ranged from 9% for L-asparaginase to 91% for hexamethylmelamine. A correlation of rate of absorption was made to their respective molecular weight, pK_a and K values. From these results recommendations can be made as to which agents, i.e., those with minimal absorption, should be employed in this adjunct mode of therapy for ovarian carcinoma. A Phase I clinical protocol is being prepared to evaluate this technique for treatment of ovarian carcinoma.

III. Contract Program

A. The Prime Contract: We have now completed three years of operation under the Prime Contract mechanism. The technical aspects of this program are working out exceedingly well. During the first contract year, the Prime awarded two subcontracts, in the second year three additional subcontracts, and two more in the third year. The major goal of the Prime Contract is the continuation of protocol toxicity studies in large animals. The Prime has provided the Division with a mechanism for rapid completion of protocol studies 40% faster than previously, with cost savings of approximately 20%. During the second and third contract years one-half the projected number of protocol drugs were available. The Prime has made more research time available to the LT scientific staff to pioneer methodology which can eventually be taken over by the Prime. For example, a rat model to assess the relative nephrotoxicity of a series of platinum analogues was developed this year and implemented (thru the Prime Contractor) at a subcontracting laboratory. This type of a developmental role is imperative in a field changing as rapidly as modern toxicology.

B. Other Contract Programs: The Laboratory of Toxicology also supervises three other contracts. One of these is in the area of drug metabolism wherein the University of Oregon is conducting studies primarily on the matabolism of nitrosoureas. Colorado State University is under contract to study the cardiotoxicity of adriamycin and has been instrumental (in collaboration with LT) in developing the rabbit model. This has been successfully implemented at a subcontracting laboratory. The third contract, with Dow Chemical Company, is involved with reproductive and teratogenic toxicity of antineoplastic agents. This resource was developed, in answer to FDA's request that such information for antineoplastic agents be part of the NDA submission. This has been a very productive contract in that in the past five years, they have completed studies for 12 agents. This information has been used or is about to be used to support NDA applications.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 03518-09 LT

PERIOD COVERED
July 1, 1976 through September 30, 1977

TITLE OF PROJECT (80 characters or less)
Pharmacologic Interruption of the Metabolism of the Dicarboxylic Amino Acids and their Amides

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

David A. Cooney, Senior Surgeon, LT, DTP, DCT, NCI
H. N. Jayaram, Senior Staff Fellow, LT, DTP, DCT, NCI
Benjamin Taylor, Jr., Staff Fellow, LT, DTP, DCT, NCI
S. Anandaraj, Fogarty Visiting Fellow, LT, DTP, DCT, NCI
Harry A. Milman, Research Assistant, LT, DTP, DCT, NCI

COOPERATING UNITS (if any) Georgetown U. (Drs. P. Schein & B. Ardalan); U. Del. (Dr. J. Wriston); Micro. Assoc. (Dr. S. Bryne); Yale U. (Dr. P. Chang): Ped. Oncology NCI (Dr. D. Poplack): LCP, NCI (Dr. R. Adamson): Mt. Sinai Hospital (Dr. H. Bruckner); LMP, NCI (Dr. M. Bradley): U. Conn. (Dr. C. Kessler).

LAB/BRANCH
LT, DTP, DCT, NCI

SECTION
Biochemistry Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, MD

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
6	5	

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Use of the oncolytic enzyme, L-asparaginase, demonstrated that deprivation of a non-essential amino acid could have lethal repercussions on tumor cells. This Section explores alternate means of achieving such deprivations by inhibiting L-asparagine synthetase, L-asparaginyl-tRNA synthetase, and stimulating endogenous L-asparaginase activities. Orthodox drugs and intermediary metabolites are among the agents being examined in a systematic way for such actions. In addition, inasmuch as L-glutamine is the ordinary source of the nitrogen of L-asparagine, we are exploring a select number of powerful L-glutamine antagonists for their perturbation of L-asparagine metabolism. The mechanisms of action of new antagonists of L-aspartic and L-glutamic acids with antitumor activity are also being studied preparatory to clinical trials with these agents. Lastly, a small number of new oncolytic enzymes are being subjected to therapeutic and toxicologic testing.

Project Description:

Objectives:

To characterize the biochemical basis for the toxicity of select oncolytic drugs and, where possible, to devise and develop antidotal measures.

Methods Employed:

Standard and experimental enzymatic, biochemical and analytical techniques have been utilized.

Major Findings:

A.1. Studies with Alanosine. Alanosine [(Propionic acid, 2-amino-3(hydroxy nitrosamino)]-DL and L (NSC-143,647 and NSC-153,353) is a new antitumor antibiotic currently undergoing preclinical toxicologic evaluation. The drug is thought to act by interrupting adenylosuccinate synthetase leading to a restriction of AMP, ATP and dATP pools. In last year's report, the fate and distribution of L-alanosine in dogs, monkeys, mice and rats was reported. Urine is the principal excretory route and a ninhydrin-negative metabolite accounts for 40 to 70% of the urinary radioactivity. Although this metabolite is electrophoretically and chromatographically identical to the α-hydroxy analog of L-alanosine, positive identification has not been made because of technical difficulties, principal among them being its resistance to derivitization.

During the past year, milligram quantities of several allied metabolites of L-alanosine have been prepared by enzymic methods and examined for cytotoxicity (ED_{50}) and for ability to inhibit adenylosuccinate synthetase [ID_{50}]: α-keto-alanosine (0.5 mM), [5.0 mM]; α-hydroxy alanosine (0.15 mM), [150.0 mM]; D-alanosine (1.0 mM), [160.0 mM]; decarboxyalanosine (0.15 mM), [150.0 mM]; desaminoalanosine (1.0 mM), [0.7 mM]; and L-alanosine (0.0005 mM), [0.6 mM]. It can be appreciated that none of these metabolites approaches L-alanosine in cytotoxic potency. This result implies either that L-alanosine itself is the proximate effector of its pharmacologic action or a minor metabolite is the responsible molecule. In this connection, it is relevant that Dr. Hurlbert, under contract to NCI, has isolated an adduct of 5-amino-4-imidazole carboxylic acid ribotide with L-alanosine, which behaves as a potent (Ki about 5μM) inhibitor of adenylosuccinate synthetase. Using adenylosuccinate synthetase from E. coli, and DEAE-Sephadex chromatography, we have isolated microgram amounts of a strongly acidic adduct of alanosine and IMP. This adduct also inhibited adenylosuccinate synthetase from mouse muscle by about 90% at a concentration estimated to be about 5×10^{-5}M. Its lability on storage even at 4° has, so far, precluded further identification. In cooperation with Dr. John Montgomery of Southern Research Institute, attempts are being made to prepare both of these adducts by chemical means. In addition, to facilitate cellular penetration, the non-phosphorylated ribosides also are being synthesized.

Influence of Alanosine on Relevant Nucleotide Pools. Extensive studies by Dr. Peter Plagemann established that L-alanosine elevated IMP and depressed AMP pools in cultured Novikoff hepatoma cells. These results are consonant with the mechanism of action postulated for the drug. We attempted to confirm these results in vivo. One hour after intraperitoneal doses of 500 mg/kg, no increase in hepatic IMP pools could be demonstrated. At the same time, however, ATP levels were reduced by 50%. Interestingly, the concentration of L-aspartic acid in liver was increased by 400% under these conditions, attesting to the functional potency of the antagonism of L-alanosine toward L-aspartic acid.

The failure of L-alanosine to provoke an increase in hepatic IMP pools is problematic and may reflect salvage routes for the utilization of IMP. Alternatively, it is possible that the alanosyl-nucleotide adducts are inhibiting adenylosuccinate lyase. Support for this speculation comes from Lowenstein's recent report that synthetic β-phosphonate analogs of succinoaminoimidazole carboxamide are exceedingly potent inhibitors of adenylosuccinate lyase. Dr. Hurlbert's adduct would be expected to be stereochemically superimposable on such a compound and to be similarly charged.

Miscellaneous Results with Alanosine and its Metabolites. In collaboration with Drs. John Olney and David Curtis, we have demonstrated that administration of L-alanosine to neonatal mice produces marked vacuolar degeneration of neurons in the hypothalamus. This effect is identical to that produced by monosodium L-glutamate and L-aspartate. The antibiotic also is a neuroexcitor to isolated cerebral neurons with a potency comparable to that of the normal dicarboxylic amino acids. Interestingly, like GABA, the decarboxylated product of L-alanosine, decarboxyalanosine is a potent neuro-inhibitory agent.

A.2. Studies with γ-Thiocyano-α-amino butyrate. In collaboration with Drs. Charlotte Ressler and Lawrence Pinkus, studies were carried out with a new amino acid, γ-thiocyano-α-amino butyrate: this compound proved to be a potent and specific analog of L-glutamine, capable of inactivating L-asparagine synthetase and carbamyl phosphate synthetase, specifically when L-glutamine as opposed to ammonia was provided as nitrogen donor. In both cases, the Ki approximated 500 μM. In addition, XMP, FGAR and PRPP amidotransferases are inhibited by the compound by about 50% at 1 mM in the presence of equimolar concentrations of L-glutamine. With L-asparagine synthetase, apparently competitive kinetics were observed with L-glutamine as the variable substrate, despite the ultimately irreversible nature of the inhibition exerted. In liver homogenate, the compound appeared to be degraded rapidly to α-amino-butyrate. It also appeared to bind non-enzymatically to sulfhydryl compounds, with the formation of cyanide and the presumed mixed disulfide of homocysteine. This may be a significant mode of metabolism in vivo. Whether a mixed disulfide can form with the reactive sulfhydryl group present in the L-glutamine binding site of many amidotransferases is under study. Noteworthy is the observation that the compound is virtually inactive as a substrate with pure glutathione S-transferase (B or E^2).

Further studies to evaluate the compound's cytotoxicity and potential as a chemotherapeutic agent are in progress.

A.3. *Studies with PALA*. PALA, [(*L*-aspartic acid,*N*-(phosphonoacetyl)-, tetrasodium salt)], NSC-224,131, inhibits L-aspartate transcarbamylase, the second enzyme in pyrimidine biosynthesis (which exists, *in vivo*, as a ternary complex with the first and third enzymes of this pathway). PALA exhibits good antitumor activity *versus* some, but not all murine solid tumors. It is the purpose of the present study to predict the toxicity of PALA to normal and neoplastic tissues on the basis of the response of the first 3 enzymes and substrates of the pyrimidine pathway to provocative (LD_{50}) doses of the drug. Miniaturized assays applicable to human biopsy material are being developed for the measurement of carbamyl phosphate synthetase (using [^{14}C] bicarbonate), aspartate transcarbamylase (using L-[4-^{14}C] aspartic acid) and dihydroorotase (using [4-^{14}C] N-carbamyl-L-aspartic acid synthesized in this laboratory). These assays have all been worked out. The measurement of the substrate pools, and techniques for the measurement of carbamyl phosphate, carbamyl-L-aspartic acid and dihydroorotic acid are still under development. The rapid flux through this pathway and the consequent low steady state concentration of these compounds is making their assessment in tumor tissues especially difficult. Tritiated L-aspartic acid of ultra-high specific activity is being used for this purpose. Analytical details are provided in a later section.

To date, it has been observed that activity of L-aspartate transcarbamylase in seven representative mouse, rat and human tumors covers a considerable span, ranging from 300 nmoles/protein/hour in L1210, to 500 nanomoles/mg protein/hr in Lewis Lung and B16, to 1000 nanomoles/mg protein/hr in P388. Despite this span, 50% inhibition was achieved *in vitro* with concentrations of PALA between 5 and 7 x 10^{-6} M, in the case of each of the tumors examined.

A.4. *Antagonists of the Metabolism of the Dicarboxylic Amino Acids - Toxicologic, Biochemical and Pharmacologic Studies*. Earlier studies on the mode of action of NSC-163,501 (L-[αS,5S]-α-amino-3-chloro-4,5-dihydro-5-isoxazoleacetic acid) indicated it to be a potent antagonist of L-glutamine. Inasmuch as this drug is scheduled to be evaluated in our toxicology program, and in order to facilitate determination of the mechanism of its biochemical toxicity in sensitive and refractory tissues, a preliminary survey was conducted in mouse organ extracts on the distribution of the target amidotransferases. This study indicated that, of the organs tested, small intestine contained only marginal concentrations of the enzyme systems utilizing L-glutamine; brain, lung, kidney and testis were rich in GMP synthetase; testes in NAD synthetase and CTP synthetase; heart, lung, pancreas, spleen and testes showed moderate PRPP amidotransferase activity, while brain and spleen were rich in N-formylglycinamide ribonucleotide amidotransferase activity; hexosephosphate aminotransferase activity was found only in liver. Further studies are being conducted to correlate percent inhibition of target enzyme(s) in an organ, with histologic damage thereto.

A.5. Azaserine. Longnecker reported that weekly treatment with moderate doses of azaserine (NSC-742) produces pancreatic tumors in rats. In order to confirm this observation, to extend it to other species and also to generate tumors of biochemical interest (e.g., tumors with enhanced levels of L-asparagine synthetase), Swiss mice and Sprague-Dawley rats were treated intraperitoneally at weekly intervals with 10 mg/kg of azaserine for 26 weeks. No significant change in the weights between the treated and control groups was observed at times up to 10 months. Histological examinations of satellite animals sacrificed after 8 months revealed no pancreatic neoplasms.

A.6.1. Studies on L-Asparagine Synthetase from Mouse Pancreas. In the Annual Report of 1976, we described the procedures for the purification of L-asparagine synthetase from mouse pancreas. Now, with the purified enzyme at hand, we attempted to delineate the mechanism of the reaction by which L-asparagine is synthesized in the pancreas, and to observe the kinetics of inhibition of the enzyme by three reversible inhibitors, aminomalonic acid, DONV and L-homoserine-β-adenylate.

Studies of the Reaction Catalyzed by Partially Purified L-Asparagine Synthetase from Mouse Pancreas. L-Asparagine synthetase catalyzes the synthesis of L-asparagine from L-aspartic acid, L-glutamine (or ammonia) and ATP-Mg. The velocity of the reaction is maximal when the $MgCl_2$ concentration is 50 mM and the ATP concentration is 10 mM. Unlike the analogous enzyme from the RADAI leukemia, L-asparagine synthetase from mouse pancreas is not stimulated by chloride ions. The partially purified enzyme catalyzes the hydrolysis of L-glutamine in the absence of any added substrate. None of the co-substrates or products of the reaction (with the exception of L-asparagine) altered the L-glutaminase activity of the enzyme. L-Asparagine completely inhibited this activity.

Partially purified enzyme preparation from mouse pancreas catalyzed an ATP-pyrophosphate exchange which was stimulated by L-aspartic acid, L-asparagine and L-glutamine. Under the conditions examined, however, an L-aspartic acid/L-asparagine or an L-glutamic acid/L-glutamine exchange could not be demonstrated.

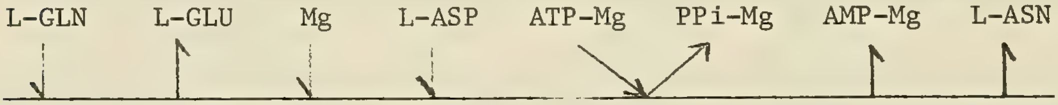
Studies of the Mechanism of the Reaction. Initial velocity and product inhibition studies were used to deduce the mechanism of the reaction catalyzed by L-asparagine synthetase from mouse pancreas.

Two parallel (L-glutamine versus ATP; L-glutamine versus L-aspartic acid) and one intersecting (L-aspartic acid versus ATP) patterns were observed in double-reciprocal plots of initial velocity studies. These results suggested a partial ping pong mechanism with a sequential addition of L-aspartic acid and ATP.

Product inhibition studies showed L-asparagine to be competitive with L-glutamine and pyrophosphate to be competitive with ATP. L-glutamic acid was non-competitive with L-glutamine; AMP and L-asparagine were non-competitive with L-aspartic acid and ATP.

The effect of $MgCl_2$ on the utilization of L-glutamine and L-aspartic acid also was investigated. In double-reciprocal plots, parallel lines were observed when L-glutamine was varied at several fixed concentrations of $MgCl_2$. An intersecting pattern (with intersection occurring on the y-axis), however, was observed when L-aspartic acid was the varied substrate.

On the basis of these results, the following reaction mechanism was hypothesized:



High (100x the Km) concentrations of ATP significantly reduced the inhibition by pyrophosphate of L-aspartic acid utilization.

Kinetics of Inhibition of L-Asparagine Synthetase from Mouse Pancreas by L-Homoserine-β-Adenylate, DONV and Aminomalonic Acid. Inhibition kinetics of three reversible inhibitors *versus* purified L-asparagine synthetase from mouse pancreas and L5178Y/AR were extensively examined.

Inhibition by L-homoserine β-adenylate was found to be dependent on the $MgCl_2$ concentration; inhibition was maximal at a $MgCl_2$ concentration of 30 mM, and an ATP concentration of 10 mM. In double-reciprocal plots, L-homoserine-β-adenylate was uncompetitive with L-glutamine and L-aspartic acid, but non-competitive with ATP. *In vitro*, the agent was very potent (> 90% inhibition of L-glutamine or NH_4Cl utilization at a 0.5 mM drug concentration).

DONV was a competitive inhibitor of L-asparagine synthetase of tumor and pancreas when L-glutamine or NH_4Cl was the variable substrates and non-competitive when L-aspartic acid or ATP was varied. This is in keeping with the structural similarity of DONV to L-glutamine or L-asparagine.

The patterns of inhibition by aminomalonic acid were rather unique. Mixed inhibition was observed when NH_4Cl or L-glutamine was the varied substrate, with inhibition at high and stimulation at low L-glutamine or NH_4Cl concentrations. Apparently, at some critical amide concentration, aminomalonic acid is able to stimulate the enzyme. Nevertheless, amidation of aminomalonic acid to yield the L-asparagine homologue, aminomalonamide, could not be demonstrated.

A.6.2. L-Asparagine Synthetase as a Predictor of Responsiveness of Acute Lymphocytic Leukemia (ALL) to Enzyme-Chemotherapy. Most cases of ALL fall into one of two classes: T-cell and null-cell. The response of these two cell types to chemotherapy (with L-asparaginase and other modalities) is radically different. It is the purpose of the present study to quantitate the levels of L-asparagine synthetase in both types of lymphoblasts separated by lymphophoresis, from prospective candidates for chemotherapy with L-asparaginase, and to correlate the results with ultimate responsiveness to the drug. Ultra-sensitive radiometric methodology is being used throughout. Ancillary pharmacokinetic (clearances) and immunologic (precipitins) techniques will be deployed in an attempt to explain the low immunogenicity of the Poplack regimen (L-asparaginase 15000 I.U./M^2 for 28 days, Prednisone 40 mg/M^2 for 28 days, Vincristine 1.5 mg/M^2 weekly for 4 weeks and Daunomycin 30 mg/M^2 3 times during this period).

In collaboration with Dr. Matthews Bradley (NCI, NIH), the effect of SV-40 viral transformation of human lung fibroblasts on the levels of L-asparagine synthetase was investigated. In two separate experiments, the transformed cell lines had increased synthetase activities (5.37 and 2.19 nmole/mg of protein/hour in the transformed cells [experiments 1 and 2, respectively] versus 0.46 and 0.64 nmole/mg of protein/hour in the native cells [experiments 1 and 2, respectively]) indicating that transformation produced a corresponding activation of synthetase activity. Now analyses are being conducted on spontaneously transformed rat fibroblasts provided by Dr. K. Sanford of NCI.

A.7. Substituted Sulfonamides and Sulfonyl Hydrazides as Potential Inhibitors of L-Asparagine Synthetase. In collaboration with Dr. Steven Brynes of the Cancer Chemotherapy Division, Microbiological Associates, Bethesda, MD, various substituted sulfonamides and sulfonyl hydrazides were prepared and tested as inhibitors of L-asparagine synthetase of the Leukemia 5178Y which is resistant to L-asparaginase (L5178Y/AR).

Of the 16 agents tested in vitro, only DL-4,4'-Dithiobis-2-[(benzyloxycarbonyl) amino] butanoic acid (Compound 1) inhibited the enzyme to a significant degree (90%) at a 10 mM drug concentration when either L-glutamine or NH_4Cl was used as amide donor. None of the 15 remaining agents significantly inhibited the enzyme at this concentration.

Compound 1 was found to be a formally competitive in vitro inhibitor of the tumoral enzyme when L-aspartic acid was the variable substrate but a non-competitive inhibitor when L-glutamine or NH_4Cl was varied.

In order to extend these observations to other enzymes acting on or producing L-glutamine or L-asparagine, Compound 1 was tested in vitro as inhibitor, at a drug concentration of 10 mM, versus several relevant enzymes. The agent was found to significantly inhibit L-glutamine transaminase (46% inhibition) but to be devoid of significant inhibitory potency against *Aerobacter* L-glutaminase (10% inhibition); mouse kidney L-glutaminase (0% inhibition); *E. coli* (pH 5.0) L-glutaminase (31% inhibition); *Erwinia* L-asparaginase (0% inhibition); *Agouti* L-asparaginase (0% inhibition); *E. coli* L-asparaginase (0% inhibition); L-glutamine synthetase (29% inhibition); XMP-dependent L-glutamine amidotransferase (0% inhibition); D-fructose-6-phosphate-dependent L-glutamine amidotransferase (0% inhibition); phosphoribosyl pyrophosphate-dependent L-glutamine amidotransferase (9% inhibition), and L-asparagine transaminase (33% inhibition).

B. Large Volume Chemotherapy of a Murine Teratoma. Elsewhere in this section of the report, a technique of large volume peritoneal lavage in the rat is described which was developed in this laboratory. This technique involves surgical procedures, making it difficult to manipulate the large numbers of animals required in chemotherapeutic studies. Therefore, a more facile procedure was developed for use in mice. The animals are given an intraperitoneal injection of drug in Hank's balanced salt solution at 25% body weight using a 27 gauge needle positioned about 8 mm above the middle of the inguinal crease. The subjects are weighed 5-60 minutes later, and a 19 gauge needle is used to make two puncture-holes about 8 mm apart in the midline near the umbilicus. The animals are returned to metabolism cages or mouse cages containing absorbant towelling and reweighed at appropriate times. Between 85%-90% of the calculated injectate is removed within 10 minutes of the performance of the second punctures; bloody taps are rare. The procedure is well-tolerated and virtually mortality free. It does provoke a vigorous diuresis.

On this background, two experimental designs are being pursued. In the first of these, the LD_{10}-LD_{50}-LD_{90} is being determined for 10 prototypical drugs - known to show some activity in human ovarian cancer or to be pharmacokinetically distinctive. Volumes of 1% versus 25% of the body weight of the recipients are used. In the latter case, the residence time of the drug in the peritoneum is 1 hour while in the former or standard case, the injectate is not removed. Five agents have been examined in this way to date: cyclophosphamide, methotrexate, cytembena, 5-FU and cis-diamminedichloroplatinum. Out of the five agents, only with platinum and methotrexate was there a discordance between the 1% and 25% body weight LD_{50}. In the case of cis-platinum, the LD_{50} and LD_{100} dose in the 1% body weight group were 15 mg/kg and 20 mg/kg respectively, while no mortalities were found in the 25% body weight even at a dose of 20 mg/kg. With methotrexate, 150 mg/kg was found to be the LD_{80} dose in the 1% body weight group and the LD_{40} dose in the 25% body weight group.

Fate and distribution studies with instillates containing radioactive drugs are planned to validate these observations.

In the second approach, C_3H mice are inoculated intraperitoneally with 1 million cells of an ascitic murine ovarian teratocarcinoma on day-zero and treated with Hank's balanced salt solution (HBSS) on day 4 as described. Subjects given the 1% body-weight injectate exhibit an MST of 14 ± 3 days while the subjects lavaged on day 4 with HBSS at 25% body-weight show an MST of 15 ± 3 days. It is proposed to examine the effect of the drugs mentioned earlier against this tumor system, as 1% and 25% body weight injectates in HBSS.

C.1. Studies with the Nitrosoureas. Dr. D. Ludlum and his group have reported that when chloroethyl nitrosoureas are reacted with polycytidylic acid, ethanocytidine is a prominent alkylation product. In order to determine whether such a tricyclic base would result from the interaction of the nitrosoureas with mammalian nucleic acids, in vitro experiments were carried out with calf thymus DNA exposed for 1 hour to chlorozotocin [alkyl-^{14}C] (1 mM) at pH 7.0. The DNA was precipitated with alcohol, redissolved in 0.01 N HCl and depurinated at 95° for 5 minutes. Alkyl products were separated by automated ion exchange chromatography as described in last year's report. Over 90% of the radioactivity was associated with a product eluting in the vicinity of 7-alkyl guanine.

This material was desalted, derivitized and examined in the mass spectrometer. Its spectrum was identical to derivitized 7-ethyl guanine. It is concluded that the production of ethanocytidine is not a major consequence of the interaction of chlorozotocin with DNA under the conditions used.

C.2. Alkylation Products of a Panel of Clinically Useful Nitrosoureas. Chloroethyl-nitrosoureas are thought to decompose to yield chloroethyl-carbonium ions. While such products could react on their own, it is reasonable to think that further decomposition could yield materials capable of being metabolized by alcohol and aldehyde dehydrogenases to chloroacetaldehyde or chloroacetic acid which in turn would be responsible for cytotoxicity. To examine if this phenomenon does occur in intact cells, murine leukemic lymphoblasts (L5178Y/AS) were treated with chlorozotocin (62.5 M) in the presence individually) of a panel of putative antidotes; 1) ethanol and acetic acid as competitive substrates; 2) disulfiram as an inhibitor of aldehyde dehydrogenase; 3) L-cysteine to trap any thiol-reactive species released during chemical decompositon of chlorozotocin; and 4) L-lysine as a trap of any carbamylating species. The results failed to indicate any protective effect of these putative counteragents under the conditions used.

D. Effect of Vitamin E on the Toxicity of Adriamycin to Mice. Adriamycin produces potentially lethal cardiotoxicity in certain strains of mice; this toxicity bears similarities to that provoked by vitamin E deficiency. Drs. Charles Myers, William McGuire, and Robert Young [Cancer Treatment Rep. 60 (7), 961, 1976] reported that α-tocopherol protected CDF_1 mice from the lethal toxicity of adriamycin. Our laboratory was charged with the duty of confirming these observations. Towards this end, two sets of experiments were conducted to examine the protective effect of vitamin E in CDF_1 mice (obtained from Flow Laboratories, Rockville, MD) versus LD_{60-90} doses of adriamycin. In the first such experiment, 0.1 ml of vitamin E (approximately 87 U of α-tocopherol of 67% purity) was administered intraperitoneally to CDF_1 mice reared on Wayne Lab Blox F6, for six weeks. Twenty-four hours later, adriamycin, 15 mg/kg, (LD of 60-90) was administered intraperitoneally; mortalities and weights were monitored. In the adriamycin group, a mortality of 60% was recorded at two weeks and there was no change in mortality at the 40th day; while in the adriamycin + vitamin E group, no mortalities were observed on the 14th day; 7% mortality was observed on the 40th day. There were no deaths at the 40th day in the vitamin E control group. In a second set of confirmatory experiments, CDF_1 mice were maintained on either Wayne Lab-Blox F6 or NIH Rat & Mouse Ration (Ralston Purina Co.) for six weeks after weaning before a similar experimental design was instituted. After two weeks, in the group of mice maintained on Wayne diet, 58% mortality was seen with adriamycin treatment (15/ mg/kg) while only 8% mortality was observed in mice treated with adriamycin plus vitamin E. However, after 40 days, 92% and 58% mortalities were observed with animals treated with adriamycin and adriamycin + vitamin E respectively. In the group of mice maintained on Purina Rat and Mouse Ration, 42% mortality was observed after two weeks in mice treated with adriamycin, while 17% mortality was seen in mice injected with adriamycin plus vitamin E. Thereafter, at 40 days a mortality of 42% and 32% was observed in groups of mice receiving adrizmycin and adriamycin + vitamin E, respectively. These experiments point to a protective effect of vitamin E against adriamycin toxicity in this strain of mice reared on Wayne diet under these experimental conditions and provide independent confirmation of the results of Dr. Charles Myers' group.

E. Analytical Methodology Under Development.

E.1. *Procedure for the Measurement of L-Glutamine Amidotransferase Activity.* In connection with studies into the mode of action of NSC-163,501 a rapid method for the estimation of amidotransferase activity was devised: 5 µl of L-[U-^{14}C] glutamine (50 µCi/ml, 200 mCi/mmole) is incubated for 30 min. at 37° with 5 µl of a crude or purified fetal liver enzyme preparation in the presence and absence of 0.01 M ATP-MgCl$_2$ and the requisite nitrogen-receptor (e.g., desamido DPN, XMP, UTP plus GTP, etc.) after which the reaction is terminated by heating the closed vessels at 95°. Any L-[U-^{14}C]glutamic acid which has been generated, then is transaminated with a large molar excess of α-ketoglutaric acid (0.01 M) at pH 8.4 in the presence of L-glutamic oxaloacetic acid transaminase. The reaction comes to equilibrium within 10 minutes at 37° after which the α-[U-^{14}C] ketoglutaric acid so produced is α-decarboxylated with 1% (v/v) H$_2$O$_2$ in 1 M HCl. The resultant [^{14}C]O$_2$ is collected in a 5 µl droplet of 40% (w/v) KOH deposited on the underside of the lids of the closed vessels.

The whole operation can be carried out in less than an hour with 50% yields of C-1. It is about six times more rapid than electrophoretic separation of precursor and product and about three times more so than decarboxylation with L-glutamate decarboxylase, an enzyme which is, at any rate, unavailable commercially in the requisite (L-glutaminase-free) purity.

E.2. *Measurement of Carbamyl Phosphate (CP) in Tumor and Normal Target Tissues.* As described earlier, an estimation of the degree to which PALA perturbs the pool(s) of carbamyl phosphate is of relevance to the ultimate activity of the drug. Measurement of this critical intermediary metabolite has been hampered by its lability and low molarity. Previous workers have been forced to include enzymic and radiochemical trapping-reagents in the medium used for homogenizing cells, and to analyze stoichiometric product (CP + ornithine→ citrulline) on the amino acid analyzer. This is a valid, but expensive and laborious approach. We have devised a more facile tactic; capitalizing on the solubility and stability of CP in ice-cold methanolic solutions, and on the availability of an alternate enzymic trap - crystalline L-aspartate transcarbamylase. In practice, liver and tumor are pressed into thin sheets between blocks of dry ice and directly homogenized in 10 volumes of methanol at -20°. The slurry is centrifuged at 12000 g for 1 min, and 500-100 µl aliquots of the waterclear supernatant lyophilized immediately. The resultant pellet is taken up in 5 µl H$_2$O and to it are added in sequence: 5µl (0.25 µCi) of L-[U-^{14}C] aspartic acid (S.A. 200 µCi/ mole) and 5 µl, 0.11 I.U. of purified L-aspartate transcarbamylase from *E. coli* in 0.05 M Tris HCl, pH 8.4. The mixtures are incubated at 37°

for 10 minutes, at which time any uncoupled L-aspartic acid is dissipated by the addition of 30 μl of 'double decarboxylation mixture' consisting of 0.5 mg GOT, 0.2 mg pyruvate decarboxylase and 0.005 M sodium α-ketoglutaric acid in 1 ml of 0.05 M sodium citrate buffer, pH 5.0. This reagent dismantles and volatilizes all 4 carbons of the L-aspartic acid molecule quantitatively. After six hours, radioactivity of the residue is measured by scintillation spectrometry. For every pmole of CP present, about 400 cpm are realized. This is very close to theory. Alkaline phosphatase blanks are included in each assay to pre-hydrolyze carbamyl phosphate. The method is reproducible and responds in a linear way to concentrations of CP between 10 and 300 pmoles. Steps are being taken to circumvent interfering materials present in biological samples: cold L-aspartic acid (which dilutes the S.A. of the L-[U-^{14}C]aspartic acid used) and alternate substrates such as formaldehyde which can be condensed with L-aspartic acid in this system.

E.3. Measurement of Carbamyl Aspartate. PALA operates by lowering carbamyl-L-aspartate pools and so terminating pyrimidine biosynthesis. It is relevant, therefore, to quantify the extent, timing, etc. of this effect in tumor and other target tissues. Colorimetric analytical methods are unsuitable for use on specimens of biological origin. We have, therefore, investigated the following approach. Duschensky *et al* observed that, whereas nitrous acid decomposes α-amino acids to the corresponding α-OH acids in an orthodox Van Slyke reaction, at pH < 1 it decarbamylates carbamyl-L-aspartic acid to the corresponding primary amino acid, stopping there. We have confirmed that 1.0 N sodium nitrite in 0.1 N HCl does convert carbamyl-L-aspartic acid in 80% yield to L-aspartic acid. We have also been fortunate to find that carbamyl-L-aspartic acid is quite soluble in cold methanol, so that extracts suitable for the measurement of CP are suitable, too, for estimation of carbamyl-L-aspartate. In practice, 500 μl of the alcohol extract is lyophilyzed and reconstituted whether with 10 μl of 1.0 N HCl or with 10 μl of 1.0 N HCl containing 0.1 M sodium nitrite. After thirty minutes, excess nitrite is destroyed by the addition of 10 μl of 1.0 M glycine acetate, pH 5, to each sample; any L-aspartic acid therein is measured via published enzymatic-spectrophotometric or enzymatic-radiometric techniques. The increase produced by nitrite, is attributed to carbamyl-L-aspartic acid. In an alternate strategy, all L-aspartic acid in the sample is removed before nitrite-treatment by means of L-aspartate-β-decarboxylase. This latter approach lowers the blank value. Using these techniques, we have not so far succeeded in measuring carbamyl-L-aspartate pools in tumor. The method, as used now, is sensitive only to about 10 nanomoles of this metabolite per aliquot, which is equivalent to a molarity of the substrate in tissue of 4×10^{-4}M.

E.4. Analytical Techniques Under Development: A Sensitive Means of Estimating Hydrogen Peroxide - Many drugs and toxins act by producing oxygen (and other) free radicals. The former is decomposed by superoxide dismutase to yield H_2O_2. Thus, it might be possible to establish the molecular mechanism of such intoxication by monitoring the accumulation of H_2O_2 in target tissues. Towards this end, a sensitive technique for estimating the presence of hydrogen peroxide is being developed. Sulfuric acid (0.1 N) extracts of flash-frozen tissues are incubated with L-[U-^{14}C]ketoglutaric acid of high specific activity for 12 hours at 37° in specially-fabricated gas bottles. During this time, H_2O_2 catalyzes the -decarboxylation of the -keto acid to yield [$^{14}CO_2$] and succinic acid. The carbon dioxide is trapped in alkali and counted by standard means. As little as 1 x 10^{-6} moles of H_2O_2 can be measured in this system. Drawbacks to its use in vivo are recognized - principal among them are vigorous autochthonous catalatic and peroxidative enzymes. Inhibitors of these are being sought. Initial studies circumvent this problem by two expedients: 1) use of acatalasemic mice; and 2) use of mice, maintained on a selenium-and vitamin E-free diet, which are devoid of cardiac and hepatic glutathione peroxidase, and also of cardiac catalase (Dr. C. Myers). These deficient strains thus serve as unnatural but useful in vivo magnifiers of H_2O_2 accumulation.

It is relevant to point out that in normal mice on ad lib Purina chow, no accumulation of H_2O_2 was demonstrable in bowel, heart, liver or kidney either at 1 or 12 hours following an LD_{60} dose of adriamycin.

E.5. Preparative Isoelectrofocusing without Carrier Ampholites. Inasmuch as proteins are ampholites, it occurred to us that isoelectrofocusing of mixtures rich in protein could be accomplished without the addition of expensive carrier Ampholines. A retrospective study of the literature reveals analogous suggestions (Svensson, Acta Chem. Scand. 15: 315, 1961).

Isoelectrofocusing was carried out on the 105,000 g (1 hr) high speed supernatants of mouse liver extract (to purify L-asparaginase), of L5178Y/AR tumor extract (to purify L-asparagine synthetase) and of bovine fetal calf serum. To 100 ml of the dialyzed supernatant 8 g of superfine Sephadex G-75 was added along with 100 µl of 2-mercaptoethanol. The resultant slurry was poured onto a rubber-gasketed glass plate, dried to obtain a matte consistency, and electrofocused at 4° by applying a current of 1500 V and 10 ma for 16 hours. Later the gel was sliced into 30 equal parts and transferred into separate tubes. Proteins from the slurry were eluted with 2 ml of water. The pH and the enzyme activities were measured in the eluate. The results indicate that multiphasic pH gradients extending between pH 3.5 to 9.1, 2.6 to 12.4 and 2.7 to 12.0 were formed in the case of tumor extract, liver extract and fetal calf serum, respectively. In addition, 5 fold purification with about 80% recovery was achieved in the case of L-asparaginase.

E.6. Separation of Metabolites of Fluorouracil. Work has been initiated to develop methods for the preparative separation of nucleoside phosphates. For such separations, a series of lithium citrate buffers with molarities of 0.015, 0.0375, 0.075, 0.15 and 0.225 respectively were utilized. All buffers were adjusted to pH 2.72 and the short column (12 x 0.8 cm) of the JEOL amino acid analyzer containing Hamilton's #78001 anion exchange resin was operated at 35°. The nucleotides of adenine, guanine, cytosine, thymine, uracil and inosine as well as derivatives of 5-fluorouracil and nicotinamide have been separated in this manner. Noteworthy is the ease with which UMP, FUMP and FdUMP are resolved.

Significance to Biomedical Research and the Program of the Institute:

Ideally, the Biochemistry unit uncovers the biochemical mechanisms underlying the toxicity of new oncolytic drugs and suggests rational antidotes for this toxicity. The stress is on enzymology, inasmuch as most drugs operate by interacting with enzymes. Practically speaking, this unit cooperates with the Laboratory of Toxicology as a whole, and with the Division of Cancer Treatment in ensuring that new chemotherapeutic modalities can be used safely in man.

Proposed Course:

It is proposed to pursue studies on new oncolytic agents analogous to amino acids, examining their toxicity at the level of enzymes, cells and in whole animals.

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 06110-07 LT

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

The Pathology of Drug-Induced Alterations in Animals and Man

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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Laboratory of Toxicology

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INSTITUTE AND LOCATION
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TOTAL MANYEARS: 4	PROFESSIONAL: 3	OTHER: 1

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The major objective of this project is to define and characterize the pathologic effects of drugs in animals and compare the findings with those reported in man. A secondary objective is to define animal models of human disease.

The specific studies involve the following:

1. The clincopathologic effects of Adriamycin in rabbits, rats and mice.
2. The effects of CoQ10, Vitamin E and Quelamycin on the Adriamycin-induced cardiotoxicity in the rabbit model.
3. The evaluation of the rat as a model for testing the nephrotoxicity of platinum containing antineoplastic agents.
4. The characterization of animal models for chondro-osseous neoplasia, hypercalcemia of malignancy and bone and joint disease.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives:

1. To define the clinicopathologic effects of adriamycin (ADR) in rabbits, mice and rats and characterize an animal model for:
   a. analogue testing
   b. antidotal testing
   c. antitumor and cardiotoxicity testing
2. To test ADR analogues and antidotal procedures in the rabbit model to determine their effects on the development of the cardiomyopathy.
   a. Quelamycin
   b. Coenzyme Q_{10}
   c. Vitamin E
3. To evaluate the rat as a model for testing the nephrotoxicity of platinum containing antineoplastic agents.
4. To evaluate model systems to study the interaction of neoplasia and bone.
   a. bone cancer
   b. skeletal metastases
   c. hypercalcemia of malignancy
5. To evaluate model systems of bone and joint disease.
   a. induced by chemotherapy
   b. inherited bone disease
   c. related to aging processes

Major Findings:

A. The Clinicopathologic Effects of ADR in Rabbits, Rats, and Mice. Recent reports of the toxic effects of ADR in animals and humans indicate that the tissues of the following eight organ systems are adversely affected: hematopoietic, lymphoreticular, digestive, reproductive, cardiovascular, urinary, skeletal and integumentary. Myelosuppression, digestive disturbances, stomatitis and alopecia are the most frequently occurring adverse effects in patients receiving ADR. It has been well accepted that the cardiotoxicity of ADR is the major total dose limiting toxic manifestation in patients. A number of animal models have been characterized to various degrees as indicators of ADR-induced cardiotoxicity. An animal model would be valuable in testing analogues of ADR for cardiotoxicity and for testing antidotal procedures. To date, only the rabbit and monkey have been reported to develop a dose-related cardiomyopathy with congestive heart failure. Smaller laboratory animals (i.e. mouse and rat) offer distinct advantages as models with respect to size, temperament, handling, care and housing and the amount of drug required for testing. Therefore, the current investigations were designed to determine if ADR would induce a significant cardiotoxic syndrome in the rat. ADR was administered to rats at doses of 1-2 mg/kg per week for 10-14 weeks. The majority of ADR-treated rats developed cardiomyopathy from 3 to 23 weeks after the last injection. Forty to seventy percent of those rats with cardiomyopathy had gross evidence of congestive heart failure (pleural effusions, ascites, hepatomegaly, cardiomegaly). Histologic myocardial changes consisted of myocyte vacuolation and degeneration, interstitial edema and mild fibroplasia. In addition, damage to atrial ganglion cells was evident in several rats. Ultrastructural alterations involved sarcoplasmic disruption with distentions of subcellular organelles and loss of myofilaments. The myocardial lesion is similar to that described in the rabbit, although it is less severe. Extra-cardiotoxic effects of ADR in the rat (nephrotoxicity, myelosuppression, enteropathy, arrested osteogenesis) were minor and observed more frequently at higher cumulative doses.

The results of the present study suggest that the rat model is an accurate, reproducible, cost effective system for large scale cardiotoxicity testing of analogues of ADR and daunorubicin (DNR).

B. The Effect of CoQ_{10} on Adriamycin-Induced Cardiotoxicity in the Rabbit Model. The present studies were designed to determine the ability of CoQ_{10} (C) to counteract the toxic effects of ADR, specifically the cardiomyopathy (CMY). Forty-four male NZW rabbits were grouped accordingly: ADR-C (12), ADR-vehicle (12), ADR-saline (12), C-saline (8). C was administered intravenously five mornings/wk at a dose of 1 mg/kg commencing five days before ADR administration. ADR was administered intravenously at a dose of 0.8 mg/kg (8.8 mg/M^2) three consecutive afternoons/wk. Vehicle and Control rabbits received similar volumes intravenously at the same schedule. ADR-C rabbits had a mean survival of 81 days, a mean total ADR dose of 305 mg/M^2 and an incidence of CMY of 83%. These findings were not significantly different from those of the ADR-Vehicle group (89 days, 336 mg/M^2, 83% incidence) or the ADR-saline group (90 days, 340 mg/M^2, 75% incidence). Semiquantitative estimation of the CMY at the light microscopic level revealed a slight reduction in the severity of the lesion in the ADR-C group compared to the other ADR treatment groups. These results indicate that C does not significantly counteract either the general toxic effects of ADR or the incidence of CMY.

C. Evaluation of Quelamycin (Triferric ADR-NSC-267,703) for Cardiotoxic Activity in the Rabbit Model. Previous reports from Professor Mario Gosalvez's laboratory suggest that Quelamycin is non-cardiotoxic based on inhibitory activity against mammalian cardiac Na-K and shark rectal gland ATPase. Four groups of six rabbits each were given Quelamycin (supplied by Montedison Farmaceutica, Barcelona, Spain) I.V. twice/week at doses 1x, 2x, 3x, and 4x the cardiotoxic dose of ADR (13 mg/M^2). Rabbits in the two higher dose groups died from toxicity at 9-23 days after initiation of treatment. Histopathologic evaluation of selected tissues revealed severe bone marrow hypoplasia and no evidence of myocardial lesions. Rabbits in the 2x group died from toxicity over a period of 14-81 days after start of treatment, at a maximum total dose of Quelamycin of 634 mg/M^2. Two of the rabbits had gross evidence of congestive heart failure at the time of necropsy. The other four rabbits exhibited diffuse petechial and ecchymotic hemorrhages on serosal surfaces of abdominal and thoracic viscera. These findings were interpreted as indications of severe myelosuppression. Selected tissues are being processed for histopathologic evaluation. One rabbit in the 1x group died of congestive heart failure at a total dose of 528 mg/M^2. The remaining five rabbits are alive and have received total doses of Quelamycin of 650 mg/M^2. These preliminary findings indicate that, (a) Quelamycin is cardiotoxic in the rabbit model, and (b) Quelamycin is approximately one-half to one third as cardiotoxic as ADR. That is to say, the critical cardiotoxic dose for Quelamycin is approximately 600 mg/M^2, whereas for ADR it is 250 mg/M^2.

Preliminary results of cardiotoxicity testing of Quelamycin in Zbinden's rat model indicate that the iron-complex is one half as cardiotoxic as the parent drug. These findings correlate well with the results in the rabbit and suggest that the qualitative and quantitative predictiveness of these two model systems are similar. The oncolytic activity of Quelamycin has been found to be less than or equivalent to ADR activity. Dr. Randall Johnson (Personal Communication) states that his findings indicate that Quelamycin is 1/3 to 1/4 as active as ADR in experimental tumor systems.

D. Evaluation of the Effects of Vitamin E on the Cardiotoxic Activity of ADR in the Rat and Rabbit. Acute Studies in the Rat - Five groups, of 10 rats each, were designated as follows: (1) ADR (I.P.) + Vit. E. (I.P.); (2) ADR (I.P.) + Saline (I.P.); (3) Vit. E. (I.P.) + Saline (I.P.); (4) ADR (I.P.) + Vit. E. (S.Q) and (5) Vit. E. (S.Q.) + Saline (I.P.). Vitamin E (Sigma No. T-3634) was injected at a dose of 3,000 I.U./kg 24 hours prior to treatment with ADR (I.P. 120 mg/M^2). The 14 day survivors for the five groups were 30%, 20%, 100%, 80% and 100% respectively. Selected tissues were fixed and processed for histopathologic evaluation. No lesions were observed in the myocardia of any of the rats. Rats receiving ADR had severe peritonitis, enteritis and lymphocytic hypoplasia of the thymus and spleen. These preliminary findings indicate that I.P. Vit. E. does not protect significantly against the lethality of I.P. ADR. Current studies are in progress to verify the apparent protection against I.P. ADR-induced lethality with prior S.Q. administration of Vit. E.

Acute Studies in the Rabbit - Six groups of 4 to 6 rabbits were designated as follows: (1) Vit. E. (I.P.) + ADR (I.P.); (2) ADR (I.P.) + Saline (I.P.); (3) Vit. E. (I.P.) + Saline (I.P.); (4) Vit. E. (S.Q.) + ADR (I.V.); (5) ADR (I.V.) + Saline (S.Q.); and (6) Vit. E. (S.Q.) + Saline (I.V.). Vitamin E (Sigma No. T-3634) was injected at a dose of 3,000 I.U. 24 hours prior to treatment with ADR (88 mg/M^2). The 14 day survivors of the treatment groups were 0%, 75%, 100%, 0%, 0% and 100%, respectively. Two additional control rabbits received 3,000 I.U. of Vitamin E. I.P. and were sacrificed 24 hours post-injection. Samples of parietal and viseral peritoneum were collected and processed for histopathologic examination. Microscopic evaluation revealed a moderate to marked granulomatous peritonitis with a predominance of mononuclear cells and neocapillary formation. Rabbits receiving ADR I.V. had severe bone marrow hypoplasia with disseminated petechial and ecchymotic hemorrhages irregardless of whether they had received Vitamin E. No lesions were evident histopathologically in any of the hearts from the test rabbits. These preliminary findings suggest that:(a) Vitamin E does not protect against the acute lethality of ADR in rabbits; (b) the rabbit peritoneum is highly sensitive to injury; (c) the preparation of

Vitamin E used is toxic to rabbit peritoneal surfaces; and (d), I.P. ADR inflicts its damage locally (peritonitis) with death occurring before any characteristic manifestations of systemic toxicity (i.e. myelosuppression) are evident.

E. Evaluation of the Rat as a Model for Testing the Nephrotoxicity of Platinum Containing Antineoplastic Agents. Nephrotoxicity is the major dose-limiting toxicity in patients receiving Cis-diamminedichloroplatinum II (NSC-119875), a new cancer chemotherapeutic drug. Experiments were designed to determine if the F344 rat would be a sensitive model for detection of nephrotoxicity of this drug and other potentially less toxic platinum containing analogues. Six agents containing platinum (NSC-119875, 250427, 239851, 180512, 224964, and chloroplatinic acid) were administered parenterally to 500 male F344 rats, 6 weeks of age, at various dose levels from LD_{10}-LD_{100}. This study showed that all six platinum-containing drugs were nephrotoxic by I.P. and/or I.V. administration. Two drugs with minimal renal toxicity by I.P. administration were highly toxic to the kidney after I.V. injection. The nephrotoxicity of chloroplatinic acid verified the potent toxicity of platinum-containing compounds to renal tubules. The most severe renal toxins (chloroplatinic acid, NSC-239851, platinum uracil blue, and NSC-250427 resulted in uremia and renal failure as the cause of death and produced necrotizing lesions of the proximal tubules throughout the entire renal cortex. The less nephrotoxic drugs [cis-DDP(II) and NSC-224964] and lower doses of the more nephrotoxic drugs induced lesions in proximal tubules of the medullary outer stripe. Similar dose response and localized toxic effects have been reported for other nephrotoxic agents. Mineralization of necrotic renal lesions was usually followed by death. The results of these studies suggest that platinum compounds should be considered as potent renal toxins along with other heavy metals.

The correlation of the elevated BUN values and histopathologic lesions in the kidney is necessary to determine the type and degree of nephrotoxicity. Previous studies indicate that histopathologic evaluation of the kidney is the most sensitive method for the determination of nephrotoxicity. Our studies support this contention. All platinum agents induced degenerative necrotizing and regenerative lesions in the proximal tubules associated with changes in BUN and creatinine levels. The results of our work and that of others indicate that the Fischer rat provides a sensitive strain for testing potentially nephrotoxic drugs.

F. Evaluation of the Ability of Furosemide to Modify the Renal Toxicity Induced by Platinum-Containing Anticancer Agents. Cis-Dichlorodiammineplatinum (II)(DDP) is a cancer chemotherapeutic agent that produces toxic effects in the hematopoietic, auditory, gastrointestinal, and urinary systems. Nephrotoxicity in man and rats appears to be the dose-limiting toxicity. Diuretics administered to patients treated with this platinum drug appeared to modify its nephrotoxicity. The present study was designed to further investigate in rats the apparent amelioration of this important toxic effect.

As early as 1-2 days after administration of a single dose of DDP in male F344 and Sprague-Dawley rats, the blood urea nitrogen (BUN) levels increased and reached a peak 4-5 days later, returning to near normal by Day 14 (2,4). The elevated BUN levels were correlated with degenerative, necrotizing, and regenerative renal tubular lesions in the pars recta of the proximal tubules. Elevated BUN levels on Day 3 were shown to be dose-related and also associated with the degree of renal damage. The results of these studies suggest that furosemide decreases urinary excretion of DDP by maintaining high plasma and renal levels of platinum at least during the first 24 hours. These studies demonstrate that furosemide pretreatment attenuates the chronic renal failure induced by weekly administration of DDP.

G. Characterization of an Animal Model for Experimental Chondroosseous Neoplasia. Osteosarcoma in humans is a highly malignant neoplasm with a poor five-year survival rate in spite of rigorous therapeutic regimens. It has been reported recently that osteosarcoma results in death within two years of onset in 4 out of 5 cases. No useful animal model has been reported for testing various chemotherapeutic modalities. Present investigations were undertaken to evaluate chondroosseous neoplasms induced by the FBJ virus in mice.

FBJ virus was injected I.P. into 145 neonatal NIH Swiss [N:NIH(s)] mice. Eighty mice developed a total of 110 neoplasms by 5 months of age. The mean latent period of the tumors was 71 days (26 to 145) postinjection. The frequency of occurrence of neoplasms at different sites was: diaphragm, 45%; ribs, 14%; vertebrae, 14%; femora, 9%; pelvic bones, 5%; tibiae, 4%; sternebrae, 3%; and inguinal area, 7%. The neoplasms were characterized histologically by elongated or rounded cells associated with an abundant connective tissue stroma. Occasional areas of bone formation and apparent osteoid metaplasia were seen. Bone tumors appeared to arise from periosteal cells, to grow by expansion, and to invade locally, but they failed to metastasize. Neoplasms of the diaphragm originated in the central aponeurosis and appeared histologically similar to bone neoplasms. Histochemical studies demonstrated abundant alkaline phosphatase in tumor cells, and ultrastructural observations revealed subcellular characteristics of osteoblasts and chondroblasts. Tumors were readily transplantable and had histopathological characteristics similar to those of the primary viral-induced tumors. The results of this study indicate that the FBJ virus induces in NIH Swiss mice a unique type of chondroosseous neoplasm derived from periosteal cells which has a resemblance to human juxtacortical (parosteal) osteosarcoma.

H. Evaluation of Model Systems to Study the Pathogenesis of the Interaction of Neoplasia and Bone. Bone is the third most common site of metastasis with disseminated osseous involvement occurring in about 80% of cancer patients during the course of their disease. Hypercalcemia is a critical clinical event which frequently is a result of the cellular/humoral interaction of neoplasia and bone. Preliminary evidence suggests that the VX-2 carcinoma in the rabbit is a good model of bone-mediated hypercalcemia of malignancy. Further experimental and clinical studies are required to elucidate the pathogenesis of skeletal metastases. To date, no animal model has been described which manifests the clinicopathologic behavior of metastatic breast cancer in women. As future model systems are explored and studies designed, the following problems will be considered: 1. Why do some cancer cells have a strong affinity for osseous tissues? 2. Why do breast carcinoma cells tend to localize in bones of the axial skeleton compared to the appendicular skeleton? 3. What role do hormones (especially female sex hormones) play in breast cancer cell predilection for osseous tissues? 4. Once breast carcinoma cells are in bone tissue what are the cellular and humoral interactions between these cells and the constituents of bone? 5. Is measurement of urinary hydroxyproline excretion a good index of active skeletal metastases?

I. Evaluation of Model Systems for Bone and Joint Disease. Bone and joint disease complex is one of the most ancient and most debilitating diseases of man. In addition, the pathogenesis of these osteoarthritic conditions is poorly understood. Recent reports indicate that avascular necrosis of bone (i.e. femoral head) has developed on occasion as a result of cancer chemotherapy. This adverse effect frequently requires surgical intervention with insertion of a prosthesis. The present studies concerning various aspects of bone and joint disease are designed to investigate the following: a) The toxic effects of an antineoplastic agent on bone and bone turnover rates; b) The feasibility of using a drug-induced osteopathic effect as a model for investigating the pathogenesis of bone and joint disease; c) The incidence and severity of osteoporosis in aging monkeys; and d), The use of genetic chrondrodystrophy in Alaskan Malamutes as a model for dwarfism in man.

J. Significance to Biomedical Research and the Program of the Institute. The need for a better understanding of the pathogenesis of anticancer drug-induced lesions in experimental and clinical studies cannot be overemphasized. The elucidation of the mechanism of ADR-induced cardiotoxicity may lead to establishment of a therapeutic or prophylactic procedure(s) to negate the cardiac failure syndrome.

Detailed characterization of appropriate experimental animal models is of paramount importance in investigative studies. The characterization of an animal model of osteosarcoma would provide a new avenue for testing various modalities against a heretofore chemoresistant tumor type. Preliminary results suggest that the viral-induced osteosarcoma in mice may be a good model for osteosarcoma in man. The caracterization of animal models of organ specific toxicity for evaluation of analogues of parent drugs is an important aspect of toxicologic studies. The results of present studies indicate that the rat is a good model for ADR-induced (chronic) cardiotoxicity and for platinum-induced renal toxicity. The VX-2 carcinoma in the rabbit appears to be a good model for osseous-mediated hypercalcemia of malignancy. Further studies are needed to characterize fully the pathogenesis of disseminated skeletal metastasis. In this respect, current studies are designed to define an animal model to investigate the "osteophilic" behavior of breast carcinoma.

K. Proposed Course. Intramural research activities will be continued and expanded under the specific objectives: a) To characterize the cardiomyopathic effects of ADR using a histopathologic-morphometric analysis system; b) To define modalities and/or analogues to alleviate the cardiomyopathic effects of the anthracycline class of agents; c) To characterize and define animal models for (1) disseminated skeletal metastases, (2) bone and joint disease; d) to develop and establish techniques in the area of quantitative morphometric analysis of tissues containing drug-induced lesions.

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 06122-05 LT

PERIOD COVERED
July 1, 1976 - September 30, 1977

TITLE OF PROJECT (80 characters or less)
Drug Interactions and Other Factors Influencing the Physiological Disposition and Toxicity of Drugs

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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TOTAL MANYEARS: 8	PROFESSIONAL: 6	OTHER: 2

CHECK APPROPRIATE BOX(ES)
☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

In recent years, it has become common practice, particularly in clinical oncology to simultaneously administer several drugs to patients for therapeutic purposes. A vast literature, both experimental and clinical, has shown that the presence of one drug may markedly influence the intensity, duration, and pharmacologic effects of another drug. Such drug interactions may have a variety of causes: they may result from alterations in drug absorption, in the binding of drugs to plasma proteins, which may directly or indirectly influence drug distribution, drug metabolism, or drug clearance via the bile or urine. Many drugs and other foreign chemical compounds known collectively as xenobiotics - are inactivated by enzymes present in liver microsomes, primarily by oxidation and conjugation. On the other hand some drugs and oncolytic agents are activated by these enzymes. Our major efforts are directed at understanding how drugs interact with each other and with biological organisms so that human drug therapy can be based on more rational grounds.

Project Description:

Objectives:

The interaction between a drug and tissue receptors which ultimately produces a pharmacologic or toxicologic response is the product of a large number of attenuating factors. For example, the distribution of drugs within the body depends upon a balance between relative organ perfusion, the affinity of the drug for binding sites within different organs, the extent of binding of the drug to plasma proteins, and the rate of its excretion into the urine and bile. Moreover, the metabolic clearance of many drugs from the body depends upon their relative rates of biotransformation to products which are more polar and more rapidly excreted. Thus, absorption, distribution, binding, metabolism and excretion tend to dictate the steady-state plasma drug concentration under specified conditions and thus determine the pharmacologic or toxicologic response. Since alterations in these factors are known to either diminish or intensify biologic responses, the need to assess these parameters quantitatively is of obvious therapeutic significance. Studies have been continued in the Drug Interactions Section to investigate and appraise these parameters in a variety of animal species regarding drugs and other xenobiotics in general, and oncolytic agents in particular. A large body of evidence has linked drug metabolism to drug disposition and the duration of drug action. Only recently have data begun to accumulate which emphasize the important role of drug metabolism or biotransformation in drug-induced toxicity. Recent findings have demonstrated that many relatively inactive foreign chemical compounds can be enzymatically activated *in vivo* to what might be termed *proximate toxicants*.

Methods Employed:

Standard enzymatic and analytical techniques have been utilized.

Major Findings:

A. Hepatic and Extrahepatic Drug Metabolism in Several Common Laboratory Animal Species. The literature contains widely discrepant reports concerning the relative mixed function oxidase (MFO) activity in hepatic and extrahepatic tissues of different laboratory species. On the one hand, very low levels of enzyme activity have been reported in the lungs and kidneys of rats, guinea pigs, and mice, while on the other hand, our laboratory and others have reported enzyme activities in rabbit lung microsomes equal to or slightly higher than the activities in rabbit liver. The present investigation was initiated in order to shed further light on this apparent species-related difference.

Comparative studies of *in vitro* drug metabolism by hepatic and extrahepatic tissues have been complicated by the use of a single experimental tissue, few animal species, and variable experimental conditions. In an attempt to minimize these complications, liver, lung and kidney from rat, mouse, rabbit, hamster, and guinea pig were assayed for standard microsomal and soluble fraction enzymes involved in drug biotransformation. For all species, liver was the most active organ (e.g., cytochrome P-450, aniline hydroxylase, aminopyrine demethylase, and N-acetyl transferases). Kidney and lung activities were usually 15%-40% of those found in liver, with kidney slightly more active than lung. No single species demonstrated total superiority in this drug-metabolizing ability, although hamster showed a large number of instances of greatest activity. The rat was a surprisingly poor representative of drug-metabolizing ability; it was superior to the other four species in less than 25% of the instances studied. All species appeared to N-demethylate aminopyrine equally except for high pulmonary and nearly absent renal activities in rabbit and high hepatic activity in hamster. Rat had the lowest level of cytochrome P-450 and low activity of NADPH-cytochrome c reductase. UDP-glucuronyltransferase activity toward the acceptors p-nitrophenol and o-aminophenol was higher in hamster and rabbit than other species. Guinea pig appeared to have the most active soluble fraction enzymes. Mouse lung and kidney had glutathione S-aryltransferase activities 10-fold greater than any other species and comparable to liver activity from rabbit and hamster.

B. Relative Effects of Various Biological Alterations on Hepatic and Extrahepatic Drug Metabolism. As part of our continuing interest in similarities and differences between drug metabolizing systems in lung and liver, various factors known to alter hepatic drug metabolism were examined for their effects on drug metabolism in certain extrahepatic organs, viz. lung and kidney. The prominent sex-related differences in drug metabolism in rat liver were not seen in either lung or kidney. Pretreatment of rats with phenobarbital produced the expected large increases in hepatic NADPH cytochrome c reductase, cytochrome P-450, aminopyrine demethylase and biphenyl hydroxylase activities without concomitant changes in any of these parameters in lung, and only scattered and smaller changes in kidney. 3-Methylcholanthrene (3-MC) pretreatment significantly increased cytochrome P-450 levels in all three organs. Pretreatment of rats with carbon tetrachloride (CCl_4) produced consistent inhibition of mixed-function oxidation in hepatic microsomes, but the extrahepatic effects were less predictable and were both organ- and enzyme-specific. An increase in renal UDP-glucuronyltransferase activity was observed after CCl_4 treatment that paralleled a similar but larger increase observed in liver. Extrahepatic NADPH cytochrome c reductase and N-methyl-p-chloroaniline demethylase values were unaffected by CCl_4. Lung and kidney responded in a like manner to liver to the additions *in vitro* of β-diethylaminoethyl diphenylpropylacetate (SKF-525A). Losses in enzyme activities in lung and kidney microsomes roughly paralleled those of liver when stored as pellets for up to 14 days at -70°. Two or four days of starvation produced substrate-specific changes in enzyme-specific activity in liver and kidney, with lung appearing resistant to the effect. When enzyme activity was expressed on a whole organ basis, however, lung cytochrome P-450 values decreased significantly and parameters from liver and kidney increased or decreased in a substrate-specific manner. It is concluded that some physiological and pharmacological factors that influence hepatic drug metabolism produce similar effects in lung and kidney, while other factors produce organ-specific effects.

C. The Effects of Ascorbic Acid Deficiency and Repletion on Pulmonary, Renal, and Hepatic Drug Metabolism in the Guinea Pig. Activities of the mixed function oxidase system and two cytosol enzymes were measured after 7, 14, 21, and 25 days of ascorbic acid deficiency, and at 3, 7, and 14 days of recovery. The ascorbic acid content of liver, lung, kidney, and adrenals decreased to less than 10% of controls during development of scurvy, and returned to control values by day 7 of the recovery period. Both cytochrome P-450 content and aminopyrine N-demethylation decreased to 60% of control values in liver microsomes at 21 and 25 days of deficiency, whereas there was no significant change in lungs and kidneys. By contrast, NADPH cytochrome c reductase did not change in any of the three organs. In the cytosol, glutathione S-aryltransferase activity was decreased in scorbutic liver, slightly decreased in kidney, and unchanged in lung. N-Acetylation of p-aminobenzoic acid was increased in kidneys of deficient animals, with no change in the other two organs. Partial recovery from decreases in drug metabolism produced by scurvy occurred by day 3 and was complete by day 7 for the microsomal enzymes. However, S-aryltransferase activity was not fully recovered even at 14 days. The already high N-acetylation activity of scorbutic kidneys increased further over controls at 3 and 7 days of ascorbic acid repletion, but decreased to control values at 14 days.

D. The Effect of Glucocorticoids on the Morphologic and Biochemical Development of Mixed Function Oxidase Activity in Prenatal Rabbit Lung. A number of published reports have indicated that treatment of prenatal rabbits with natural (cortisol) or synthetic (9 α-fluoroprednisolone) adrenal glucocorticoids accelerates or causes premature:

1. Morphologic maturation of lung as determined by light microscopy.
2. Preferential maturation of pulmonary type II cells, particularly the cytoplasmic structures as revealed by electron microscopy.
3. Increases in pulmonary surfactant content.

Taken together with other information, these observations suggested the possibility that prenatal corticosteroids might result in a premature induction or appearance of pulmonary enzymes involved in drug metabolism. Experiments are presently underway to confirm the reported morphologic and biochemical effects of cortisol injected into the fetuses *in utero* and data on possible effects on pulmonary drug metabolism should be forthcoming very soon.

E. Effects of Ascorbic Acid Supplementation on Hepatic Drug Metabolism in Guinea Pigs. Deficiency of ascorbic acid has been repeatedly shown to impair hepatic microsomal drug metabolism and to prolong the action of certain drugs. Conversely, recent studies have suggested that ascorbic acid supplementation might increase drug metabolism to above control levels in a roughly dose-dependent fashion, but because of the design of these experiments, the results are difficult to interpret. The purpose of the present study was to determine whether maintenance of animals on diets which differed only in their content of ascorbate would affect levels of hepatic drug metabolism. Guinea pigs were maintained on four diets which contained 0.3, 1.5, 3.5, and 7.0 mg ascorbic acid per g of diet. The lowest diet contained sufficient ascorbic acid to prevent scurvy, while the two diets highest in ascorbic acid content produced maximum hepatic ascorbate concentrations after eight weeks. Several hepatic enzymes were measured after 1, 2, 3, 4, and 8 weeks. There were no significant differences among the four groups in the following parameters of microsomal mixed function oxidation: cytochrome P-450 content, NADPH cytochrome c reductase, and aminopyrine N-demethylase. Also, there were no differences in native and nucleotide-activated UDP-glucuronyl transferase activities. However, in the post-microsomal supernatant, overall glutathione S-aryl transferase activity was significantly lower in the group with the lowest supplementation of ascorbic acid, as compared to the three higher groups. Kinetic parameters of aminopyrine N-demethylation were studied in animals maintained for 21 days on a diet deficient in ascorbic acid ($<$ 0.1 mg ascorbic acid/g), an intermediate control diet (1.5 mg ascorbic acid/g) and a high-dose ascorbic acid diet (7.0 mg ascorbic acid/g). Curvilinear Lineweaver-Burk plots were obtained in all three groups, and apparent K_m and V_{max} calculated separately for both low and high ranges of substrate concentrations. Apparent K_m's did not differ significantly among the three groups. Microsomes from ascorbic acid deficient animals had V_{max} values lower than the control and high-dose groups. There was no difference in kinetic parameters between microsomes from animals receiving control and high-dose ascorbic acid diets.

F. Studies on the Possible Role of Protein Synthesis in Cell Injury by Free Radical Pathogens. Several theories have been advanced in attempts to understand the subcellular processes terminating in chemically-induced cell death but none is entirely satisfactory. Alterations once considered as critical to explain cell necrosis were later found either insufficient to account for the effects or not to be general features in the action of very toxic compounds. It may very well be that no unifying mechanism of cellular necrosis is operative and that no single alteration in a given organelle may by itself provide a common pattern for necrosis produced by different toxic compounds. In recent years, however, it has become apparent that protein synthesis may play an active role in the production of cellular damage. This possibility arose from experiments in which inhibitors of protein synthesis were found to partially prevent chemically-induced cellular necrosis as well as some other manifestations of the tissue damage caused by several cytotoxic agents in the rat liver and pancreas. The question arose as to whether the protective effect of these inhibitors was related to their activity as inhibitors of protein biosynthesis, per se, or to some non-specific, indirect effect. One of the inhibitors of protein synthesis frequently employed in such studies was cycloheximide (NSC-185). In the present work we sought to discover if cycloheximide administration had any effect on parameters known to be modified during CCl_4-induced liver necrosis. Cycloheximide pretreatment (1 mg/kg i.p.) slightly attenuated the hepatic necrosis 24 hr after CCl_4 administration and this attenuation was more obvious at 72 hr. A larger dose of cycloheximide (2 mg/kg) evoked a marked antidotal effect even at 24 hr.

Prior treatment of the rats with cycloheximide (1 mg/kg) decreased the amount of irreversible binding of $^{14}CCl_4$ to liver microsomal lipids and reduced

CCl_4-induced lipid peroxidation at 1 hr but not at 6 hr after administration of the antidotal drug. Cycloheximide did not alter the pentobarbital sleeping time of the rats nor did it interact with microsomes to give spectral changes. Cycloheximide did not alter microsomal cytochrome P-450 reductase activity. Administration of cycloheximide prior to CCl_4 did not prevent the CCl_4-induced decrease in cytochrome P-450 or glucose 6-phosphatase activity caused by the hepatotoxin, however it did prevent the CCl_4-induced polysome breakdown and it did attenuate the severity of the lesions caused by CCl_4 at ultrastructural level. The results are consistent with the view that the protective effects of cycloheximide may be indirectly or even unrelated to its effects on protein metabolism, but may instead be related to an effect on the metabolic activation of CCl_4 to CCl_3 free radical, the putative proximate toxicant.

G. Pulmonary Fibrosis Associated with Bleomycin Administration. It is generally recognized that the primary, dose-limiting toxicity of this valuable antineoplastic agent is its effects on the lung; one observes an acute inflammatory response, and interstitial edema which progress in the final stages to pulmonary fibrosis. Other useful oncolytic agents such as busulfan and methotrexate as well as a number of other xenobiotics are known to produce similar forms of pneumotoxicity.

An interesting and unique property of mammalian lung is its ability to concentrate and tenaciously retain basic amine xenobiotics, particularly those with pK_a's > 8.5. In some cases, tissue:plasma ratios range from 10-20:1. The affinity of different compounds for the binding sites in lung varies somewhat and accordingly, one drug may be released by another of higher affinity or uptake may be blocked. Our initial experimental hypothesis was predicated on the following facts and assumptions:

1. That bleomycin is concentrated to some extent in lung relative to plasma and other organs.

2. That a reduction in pulmonary uptake of the drug would be associated with a decrease in the incidence of its pneumotoxicity.

3. That the acute inflammatory effect might be blocked or reduced by anti-inflammatory drugs such as adrenal glucocorticoids.

4. That the fibrosis might be amenable to blockade by inhibitors of collagen biosynthesis such as acexamic acid.

Mice were pretreated with several doses of imipramine or diphenhydramine, two compounds known to be highly concentrated and tenaciously retained by lung, and at various times thereafter received ^{125}I-labeled bleomycin. Under the conditions employed, contrary to the hypothesis, neither amine significantly reduced the pulmonary uptake of radiolabeled drug. We have planned experiments to determine whether basic amines can displace bleomycin from lung once it is present; whether the dosage schedule of the amine (single acute dose, subacute, chronic) may influence the results; and whether decreasing the pulmonary levels of bleomycin does, in fact, decrease its pneumotoxicity. In addition, using total lung hydroxyproline as a biologic marker of collagen content (and thus, fibrosis), preliminary data indicate that this is an earlier and more sensitive index of fibrosis than other criteria that have been previously examined. We hope in the near future to examine bleomycin A_2 and two chemical congeners for their relative effects upon the lung. Quantitative morphometry, to be provided by Dr. David Young of the Comparative Pathology Section, Laboratory of Toxicology, will be used to confirm biochemical evidence of fibrosis.

H. Effect of Hyperthermia on the Disposition and Metabolism of Adriamycin. As a result of increasing clinical interest in hyperthermia, either alone or in combination with other therapeutic modalities, we have recently completed a thorough examination of the effects of hyperthermia on adriamycin disposition and metabolism in rabbits. Normothermic rabbits and hyperthermic rabbits had rectal temperatures of 39.6 ± 0.2 and 42.5 ± 0.2°C respectively during one hour following adriamycin administration. Minimal differences were found between the two groups in plasma decay, tissue distribution, metabolism and excretion of adriamycin. Administration of adriamycin to both normothermic and hyperthermic animals caused a 3-4 fold increase in serum CPK levels.

The results of the above experiments prompted us to initiate a study of the effects of temperature on microsomal adriamycin and adriamycinol reductive glycosidase and cytosolic adriamycin keto-reductase activities using ^{14}C-adriamycin. These experiments are currently in progress.

I. Uptake of Basic Drugs into Normal Lung or Lung Tumor. As pointed out above, mammalian lung concentrates basic drugs against a considerable concentration gradient. Although little or nothing is known about the physicochemical mechanisms involved in this process, we wondered if lung tumors shared with pulmonary tissue, this unique capability. We are currently investigating the relative abilities of mouse lung and Lewis lung carcinoma (LL) to concentrate a model basic drug, imipramine. If pulmonary tumor tissue retains the ability of normal lung to concentrate basic drugs, the basicity of a potential new oncolytic agent might be utilized as a delivery system to concentrate it in lung, lung tumor, and extrapulmonary metastases. Mice bearing subcutaneously implanted LL were injected with the model basic compound, ^{14}C-imipramine and at various times thereafter, groups were sacrificed and serum, lung, tumor and other organs examined for total radioactivity, and for parent drug and major metabolites. Experiments of this sort were performed at four times after tumor implantation in order to evaluate possible effects of tumor growth or size on uptake. At early times after tumor implantation (6 and 10 days), the uptake of radioactivity from ^{14}C-imipramine by tumor was low but at later times (15 and 20 days) the tumor and lung had equivalent affinities. The lung:plasma ratio for imipramine was much lower in the (control) mouse than has been reported previously for the rat (e.g. 2-3 for mouse, > 10 for rat). It is not clear whether this reflects a species difference in uptake and binding or in metabolic profiles. Experiments to answer this question are currently in progress. We also plan to investigate drug metabolism in the LL as well as uptake and efflux of drugs and other xenobiotics by normal lung and tumor slices *in vitro*.

J. Disposition of Cis-diamminedichloroplatinum (CDDP). In conjunction with the Biomedical Engineering and Instrumentation Branch, Division of Research Services, we have investigated the disposition of CDDP following its intravenous injection (1 mg/kg) in beagle dogs, sharks and rats.

Plasma levels of platinum in dogs showed a distinctly biphasic clearance pattern with estimated half-times of about 20 minutes and nearly five days. During the first four hours after treatment, plasma levels fell by 90% and 60-70% of the administered dose was recovered in the urine. Platinum was still detectable twelve days after treatment, with no significant change in plasma concentration after the fourth day. Initial concentrations of platinum were highest in organs of excretion, in gonads, spleen and adrenals but remained significantly elevated only in kidney, liver, ovary and uterus, where tissue: plasma ratios of 3-4 were maintained for as long as six days post-treatment.

Following i.v. administration of CDDP to male rats, platinum plasma levels also had a biphasic half-time with an initial phase of about 40 minutes and a long phase of two days. Small quantities of platinum were detectable after 48 hours. Large quantities of platinum were recovered in urine during the first 48 hours post-treatment. Organ distribution was similar to that in the dog, with high initial and persisting levels of platinum found in liver, kidney and gonad.

The dogfish shark exhibited the slowest rate of clearance from the blood, with an initial $T_{1/2}$ of nearly 70 minutes and a long phase $T_{1/2}$ of about 10 days. Six days after treatment, only 10% of the administered dose had been recovered in the urine. Shark kidney and uterus contained large and persisting amounts of platinum, but the liver, in contrast to the other two species, contained only small amounts of platinum.

K. Comparison of Drug Toxicity and Drug Metabolism in Several Primate and Subprimate Species. It has been recognized for at least five years that looming on the horizon was an impending under-supply of rhesus monkeys for use in biomedical research. Given the heavy reliance that has been placed on the rhesus monkey in drug-development programs and particularly in the development of oncolytic agents, it was deemed desirable to attempt to identify an alternative species to the rhesus for possible use in the Cancer Program. In conjunction with Battelle Columbus Laboratories (BCL) through the Toxicology Program Office (TPO), personnel from the Drug Interactions Section of the Laboratory of Toxicology devised an experimental design to attempt to identify such a species. Five animal species were selected for study and in order to accommodate possible sex differences, both males and females were scheduled for study. The species were: rhesus monkey, squirrel monkey, tupaia (a primative primate species), mini-pigs and rats. The design involved three major subdivisions, each of which could be pursued independently, yet the data could be finally assembled in a way that all three components could be compared. The subdivisions were: (1) protocol toxicologic evaluation of three selected antineoplastic agents (Adriamycin, Ara C, Cytoxan), (2) in vivo metabolism of the same three oncolytic drugs (to provide data on qualitative differences in drug metabolism), and (3) in vitro metabolism of a number of model drug substrates by hepatic preparations, both microsomal and post-microsomal supernatant fractions (to provide data on quantitative differences in drug metabolism). The experiments on points 1 and 2 above were conducted exclusively by BCL employees whereas the experiments in part 3 were conducted jointly by BCL and LT personnel at the BCL facility.

Fourteen parameters of in vitro drug metabolism by hepatic microsomal and soluble fractions were examined in adult male and female rhesus monkeys, squirrel monkeys, Hanford miniature pigs, common tree shrews and Sprague-Dawley rats. All species tested demonstrated activity in all but one test and all showed some similarity to the rhesus. None of the species, however, was totally comparable to the rhesus in drug metabolizing ability. The squirrel monkey showed the least similarity to the rhesus in drug metabolizing ability, and the miniature pig was the most similar. With the exception of the expected differences in the rat, the tree shrew demonstrated the only sex difference in drug metabolism, the enzyme activities of females being higher than the male in several pathways. The data suggest that any of the four species tested could be a suitable replacement for the rhesus in studies of in vitro drug metabolism. Awaiting completion of these studies, a preliminary impression suggests that the mini-pig may most closely resemble man in these parameters.

L. Effect of Obesity on Drug Metabolism in Rodents. Literature reports have suggested that differences in drug response between obese and non-obese patients might be related to differences in microsomal drug metabolism. The project is nearly complete and shows MFO values in obese rodents to be only 20-30% of control values. Hexobarbital sleep times are nearly twice as long in the obese animals and hexobarbital blood levels at awakening are equal in control and obese rats. Obese rats appear to respond to the inductive effects of both phenobarbital and 3-MC in a normal manner.

M. Xenobiotic Pharmacokinetics in Marine Species. For the past several years we have been exploring the possibility of using the dogfish shark as a model species for the rapid accrual of pharmacokinetic information concerning antineoplastic agents and other xenobiotics. The major rationale for this approach has been that both the rate of metabolism and rate of disposition of compounds administered to these animals might be lower than that of mammals. Thus, in pharmacokinetics, as in other areas of pharmacology, considerably more attention is focused on the extrapolation of results from animals such as rodents, dogs, primates, upwards to man at the top of the phylogenetic hierarchy. Scaling processes also should be applicable in the opposite direction, i.e., from intermediary species to lower species. The validity of pharmacokinetic modeling in aquatic animals has been tested in only very few instances despite the availability of appropriate experimental methodology. Furthermore, there is clearly a need to discover how marine animals in their unique environment handle the increasing number of xenobiotics to which they are exposed. In addition, certain types of intact-animal pharmacokinetic studies may be performed more econocically in marine species. In order to understand the mechanism and determine the kinetics of underlying pharmacokinetic phenomena, it is often important to study events occurring in distribution phase for administered agents. In mammalian species the circulation time (blood volume/cardiac output) is typically small compared with practicable sampling intervals. Consequently, in mammals the distribution phase for water-soluble agents often occurs within the first few time points. Resolution may be improved by using a marine species with a relatively longer circulation time.

In one study we examined a candidate species, the dogfish shark (Squalus acanthias), which exhibits a circulation time of the order of three minutes. A multicompartment model was utilized to simulate the time course of distribution and excretion of a well-studied model compound, phenol red. A flow-limited multicompartment model was developed to simulate the distribution and disposition of phenol red in the dogfish after intravenous administration. Glucuronide conjugate, as well as parent compound, was found in urine and bile, but not in plasma, kidney, or liver tissue. An apparent four-hour lag in phenol red appearance in the gallbladder was simulated using two stirred tanks connected in series to represent the bile ducts. The model was extended to a number of antineoplastic agents (ca. 30) where emphasis was placed on the major routes of excretion, tissue distribution and central nervous system penetration. Plasma half-times for 50% of the drugs studied were virtually the same as in the human and for the others, no shark $t_{1/2}$ value differed from that of the human by more than 2-fold. Considering the huge phylogenic jump between man and shark, this is an excellent correlation. Relative dominance of urinary vs biliary excretion of anticancer drugs in the shark appeared to follow the trends reported for mammals; analysis of data for this portion of these studies are incomplete. Research of the central nervous system compartment of the shark has shown that not only is the methodology quite facile with regard to sampling and modeling, but it appears to be physiologically similar to that of higher vertebrates. Of a variety of central nervous system parameters studied in the shark, correlations were best when 24 hr brain/plasma ratios were compared with the results of the mouse ependymoblastoma model. Thus, if this ratio was 0.8 or greater in the shark, then usually the mouse screen was positive. From a Program point-of-view, it must be indicated that for the three highest brain/plasma ratios seen in the shark, alanosine (44), ellipticine (33) and hycanthone (10), all three compounds were positive in the tumor model and hence, should be considered as candidates in human trials for central nervous system tumors.

N. Transport of Model Compounds and Antineoplastic Agents Across the Rat Peritoneum. We tested the feasibility of using intraperitoneal antineoplastic agents containing higher than therapeutically active doses, the hypothesis being that the entire peritoneal surface would be bathed in drug solution while systemic absorption and its potentially lethal side-effects would be minimized. This mode of treatment could serve as an adjunct therapy to surgical resection in the earlier stages of ovarian carcinoma which are characterized by microscopic intraperitoneal metastases.

The transport of various model compounds and antineoplastic agents across the peritoneum of the rat was investigated to assess the influence of three major parameters on their absorption into the systemic circulation: 1) molecular weight; 2) the dissociation constant (pK_a); and 3), the lipid-water partition coefficient (K). Sprague-Dawley rats were injected intraperitoneally with radiolabeled model compounds (25) and antineoplastic agents (13) in large volumes of normal saline (50 ml). After one hour, the peritoneal fluid was removed and the absorption and tissue distribution of the compounds was measured.

The 9 neutral model compounds tested ranged in molecular weight from 18 to 2 million; their peritoneal absorption varied from 69 to 2% respectively. Five compounds were investigated with variable K values in heptane (0.001-10); their absorption ranged from 57-96% respectively. A series of 10 acids and bases covering a wide range of pK_a values were examined; for the acids, absorption increased with increasing pK_a values, while for bases the reverse appeared to be true. The absorption characteristics of 13 antineoplastic agents were determined; their absorption ranged from 9% for L-asparaginase to 91% for hexamethylmelamine. A correlation was made to their respective molecular weight, pK_a and K values. From these results recommendations can be made as to which agents, i.e. those causing minimal absorption, should be employed in this adjunct mode of therapy for ovarian carcinoma.

O. Drug Metabolism in the Nude Athymic Mouse. Present indications are that the heavy reliance that has been placed in the past on the L1210 and B16 melanoma systems in screening for potential new antineoplastic compounds will be supplanted in part by explants and transplants of human and animal tumors. The host organism for these tumors will be the nude, athymic mouse whose aberrant immunologic system does not reject these heterologous tumors. Knowledge of the ability of this mutant mouse strain to metabolize drugs was therefore of obvious importance.

Liver microsomal and supernatant fractions from male and female athymic nude mice (nu/nu) of the NIH Swiss background were studied for their ability to metabolize standard drug substrates in vitro. Controls were homozygous NIH Swiss mice (++) and mice heterozygous for the athymic gene (nu/+). The following enzyme activities were higher in the male athymic animals than in controls: NADPH cytochrome c reductase, ethylmorphine and aminopyrine N-demethylase, native OAP glucuronyltransferase and glutathione S-aryltransferase. No difference in activity between the athymic mice and controls was seen with UDP-NAG-activated OAP-glucuronyl transferase, PABA N-acetyltransferase, aniline hydroxylase, or in the amounts of cytochrome P-450. Liver:body weight ratio was slightly higher in the athymics than in contols (5.9 vs 5.2%) but microsomal protein yield was less (26.7 vs 32.9 mg/g).

In the female, only the N-demethylase pathway with both substrates was significantly elevated in the athymics relative to controls. Athymic mice responded quantitatively and qualitatively like the controls to pretreatment with either phenobarbital or 3-MC. Enzyme activities from heterozygous mice segregated into 3 separate catagories relative to the controls and to the homozygous recessives. The data are consistent with a hypothesis that MFO activity may be passed genetically as a single allele that undergoes traditional dominant-recessive expression.

Significance to Biomedical Research and the Program of the Institute:

As stated under objectives, drug interactions are known to profoundly influence the disposition of drugs and thus may determine whether a normal therapeutic dose of drug will become either a sub-threshold or toxic dose. The significance of this knowledge to cancer chemotherapy, particularly in the era of combination drug administration, cannot be overemphasized. The studies on peritoneal transport have great potential in the treatment of ovarian cancer.

Proposed Course:

The projects being pursued are only partially complete. In addition to examining in more detail the influence of antineoplastic agents upon the metabolism, distribution, and binding of drugs and their toxicity, we envision studying the metabolic fate of antineoplastic agents. Pharmacokinetic studies of the dogfish shark model have only begun and will continue in greater detail, particularly for those anticancer agents just entering clinical trials.

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ANNUAL REPORT OF THE LABORATORY OF TUMOR CELL BIOLOGY

DEVELOPMENTAL THERAPEUTICS PROGRAM

NATIONAL CANCER INSTITUTE

July 1, 1976 - - September 30, 1977

This Laboratory is concerned with six major areas of research: (1) molecular and physiological control mechanisms in normal and neoplastic cells, designed to obtain information on the molecular mechanisms involved in neoplastic transformation, including a search for viral genomes and genome products in human tumor tissues; (2) the identification, isolation and demonstration of biological activity of viral information in human leukemic cells; (3) search for biochemical markers of minimal neoplastic disease and the development of practically useful microtests for the detection of such markers; (4) cell differentiation *in vitro*. (This relates to a major interest of the laboratory: Does the phenotypic abnormality of leukemia in man result from a block in leukocyte maturation?) (6) Based on new information in the literature and from studies within this Laboratory, new approaches to cancer chemotherapy are evaluated in *in vitro* and *in vivo* systems.

During the past year a number of findings were reported by investigators from the Laboratory:

1. Established in culture a continuously replicating leukocyte line from the blood of a patient with acute promyelocytic leukemia. After initial stimulation by conditioned media from a cultured human embryonic lung, this line is able to grow in the absence of conditioned media and shows continued evidence of myeloid differentiation.

2. One isolate of HL23 virus (derived from the leukocytes of a patient with acute myelogenous leukemia) has been shown to possess fibroblast transforming activity *in vitro*. This is an important observation because it could mark the isolation of transforming genes of human origin.

3. Isolation of conditioned media from PHA stimulated lymphocytes which promote the growth of T lymphocytes.

4. Detection of proviral DNA sequences related to the baboon endogenous virus and Rauscher murine leukemia virus in fresh tissues of some leukemic patients.

5. Demonstration that leukemic leukocytes synthesize one or more species of high molecular weight type C RNA tumor virus like RNA (50-70S), while bone marrow or blood leukocytes from normal donors do not.

6. A technique has been developed for the enrichment of type C viral sequences of high G+C content by banding of DNA in sodium iodide gradients. This technique will be useful in enriching of high G+C containing proviral sequences in human leukemic tissues for molecular hybridization studies.

7. A technique of competitive RNA hybridization has been developed for the detection of viral related RNA in leukemic tissues. Preliminary results indicate the presence of mammalian type C virus related RNA in tissues of some leukemic patients.

8. A technique of in situ hybridization of viral cDNA to RNA of fixed cells has been developed for the detection of type C RNA tumor virus RNA in cells producing virus. This technique will be extended to the detection of viral related RNA in human leukemic cells.

9. A new isolate of a primate type C virus has been obtained from tissues of a gibbon with acute malignant lymphoma and leukemia. This gibbon was obtained from Hall's Island, Bermuda. The new virus isolate is designated $GaLV_H$, and has biochemical properties similar to the other known isolates from gibbons.

10. Isolation of antibodies, from short term cultures of leukocytes from CML patients, that possess high neutralizing activity against feline and murine leukemia virus reverse transcriptases. Antibodies isolated from AML patients possess high activity against reverse transcriptase from HL-23 virus.

11. Purification of DNA polymerase gamma to homogeneity and preparation of antibody against this enzyme. This antibody will be useful in distinguishing viral reverse transcriptase and cellular DNA polymerase γ, since both of these enzymes can utilize $(dT)_{15}\cdot(A)_n$ as primer-template.

12. Development of a sensitive double antibody precipitation technique for the detection of cross reaction of antibodies with reverse transcriptase and DNA polymerase γ.

13. Demonstrated the presence of terminal deoxynucleotidyl transferase in CML patients in acute blast phase of the disease, its loss during treatment, and reappearance just before blast crisis. This enzyme is a potential biological marker for following these patients during treatment and remission.

14. Showed by cell separation studies that terminal deoxynucleotidyl transferase is present in a subpopulation of cells in normal bone marrow.

15. Demonstrated the association of reverse transcriptase with plasma membranes of virus transformed cells as well as from a patient with acute lymphocytic leukemia.

16. Demonstrated the presence of inhibitors on the plasma membranes from some leukemic patients that specifically inhibit simian sarcoma virus reverse transcriptase.

17. Optimized conditions for the preparation of large cDNA copies from primate type C viruses for restriction enzyme analysis, and for molecular hybridization studies.

18. Purified to near homogeneity the glycoprotein gp70 and p30 of simian sarcoma virus and raised antisera against p30. Preparation of antisera against the gp70 is in progress.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 06117-05 LTCB

PERIOD COVERED

July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Molecular and Physiological Control Mechanisms in Normal and Neoplastic Cells

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Robert C. Gallo	Chief, Lab. of Tumor Cell Biology	LTCB	NCI
Others:	Robert E. Gallagher	Senior Surgeon	LTCB	NCI
	Prem S. Sarin	Chemist	LTCB	NCI
	David Gillespie	Microbiologist	LTCB	NCI
	W. Carl Saxinger	Microbiologist	LTCB	NCI
	Frank Meyskens	Clinical Associate	LTCB	NCI
	David Strayer	Clinical Associate	LTCB	NCI
	M. Virmani	Visiting Fellow	LTCB	NCI
	Flossie Wong-Staal	Cancer Expert	LTCB	NCI
	Marjorie Guroff	Cancer Expert	LTCB	NCI
	H. Nakazato	Cancer Expert	LTCB	NCI
	Richard A. Knight	Cancer Expert	LTCB	NCI
	Bert Vogelstein	Research Associate	LTCB,	NCI

COOPERATING UNITS (if any) Richard Adamson, Laboratory of Chemical Pharmacology, National Cancer Institute; George Todaro, Viral Leukemia and Lymphoma Branch, National Cancer Institute; Jeff Schlom, Breast Cancer Segment, National Cancer Institute; Stu Aaronson, Viral Carcinogenesis Branch, National Cancer Institute;

LAB/BRANCH

Laboratory of Tumor Cell Biology

SECTION

Section on Cellular Control Mechanisms
and Section on Nucleic Acid Hybridization

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
31	15	16

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This Laboratory is concerned with five areas of research: (1) molecular and physiological control mechanisms in normal and neoplastic cells, designed to obtain information on the molecular mechanisms involved in neoplastic transformation, including a search fro viral genomes and genome products in human tumor tissues; (2) the identification, isolation and demonstration of biological activity of viral information to human leukemic cells; (3) search for biochemical markers of minimal neoplastic disease and the development of practically useful microtests for the detection of such markers; (4) cell cycle characteristics; (5) biochemical and physiological studies on leukocyte maturation in vitro. (This relates to a major interest of the laboratory: Does leukemia in man result from a block in leukocyte maturation?) (6) Based on new information in the literature and from studies within this laboratory, new approaches to cancer chemotherapy are evaluated in in vitro and in vivo systems. This is the ultimate goal of the Laboratory.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

Richard Guy Smith	Senior Staff Fellow	LTCB	NCI
Steven J. Collins	Clinical Associate	LTCB	NCI
Rajen Koshy	Visiting Fellow	LTCB	NCI
Paul Jacquemin	International Fellow	LTCB	NCI

COOPERATING UNITS

Tadao Aoki, Viral Leukemia and Lymphoma Branch, National Cancer Institute; Sol Spiegelman, Columbia University College of Physicians & Surgeons; Robert Ting, Biotech Laboratories; Rolf Neth, University of Hamburg; Natalie Teich, Robin Weiss, Imperial Cancer Research Fund, London, England; Michael Hanna and James Ihle, Frederick Cancer Research Center; Fritz Deinhardt, University of Illinois; Wolfe Prensky, Sloan-Kettering Institute; Ray Gilden, Frederick Cancer Research Center; Dani Bolognesi, Duke University; Ken McCredie, M. D. Anderson Hospital and Tumor Institute; Marcel Baluda, University of California at Los Angeles; Richard Metzgar, Duke University Medical Center.

Project Description:

Objectives

1. It is anticipated that a greater understanding of the processes involved in the molecular control of cellular growth, differentiation, and carcinogenic transformation, including the pathogenesis of human neoplasias, will lead to the ultimate goal of developing improved approaches to therapy of human neoplasia.

2. The development of "markers" of neoplastic cells may lead to (a) quantitation of residual tumor cells after therapy and (b) determining whether cells (e.g., in leukemia) of patients in remission are really normal.

3. To develop new concepts of chemotherapy and apply them to animal model systems as rapidly as possible as new information is derived from basic experimental studies.

These objectives have primarily been pursued by the following approaches:

1. Purification and properties of enzymes of nucleic acid metabolism especially DNA polymerases (DNA-dependent and RNA-dependent) of human normal and leukemic cells and of RNA oncogenic viruses to determine ultimately:

    a. The function, characteristics, and number of DNA polymerases in these cells and viruses.

    b. If RNA-dependent DNA polymerase is present in human leukemic cells and, if so, is it the same as the virion enzyme? Can it copy viral 70S RNA? Can it copy cellular messenger RNA? Does it function in gene amplification during cellular differentiation? Is its activity altered in leukemogenesis? If found, is its activity necessary for maintenance of the neoplastic cell? Can it be used as a marker of neoplastic cells? If it is not necessary for maintenance of the neoplastic state will its continued inhibition prevent some relapses of animals or patients in remission, i.e., does the inciting agent in human leukemia (? virus) remain and produce subsequent transformation?

    c. Is the viral enzyme necessary for viral replication and for initiation of transformation?

Several of the above questions have now been answered.

2. Biochemical studies on the properties of the RNA of type-C viruses and on the overall pathway of replication of these viruses. Purposes:

    a. To obtain more information on the mechanism of transcription of this RNA to DNA via reverse transcriptase.

   b. To determine if diagnostic probes can be obtained, i.e., is their structure specific enough that we can use this information to find viral RNA in cells?

   c. In understanding the mechanisms involved in integration and expression of viral genes, we can plan approaches to interfere with this expression and then evaluate the overall biological effect of this interference. We particularly wish to know if viral expression is required to maintain the cell in the neoplastic state.

3. Pursuing studies leading to an understanding of the origin of tumor viruses, how they acquire their oncogenic potential, how they interact with cells, and how they are transmitted throughout nature. These studies are primarily carried out with techniques of molecular hybridization.

4. Leukocyte differentiation *in vitro*. The soft agar technique for investigating maturation and proliferation of normal and leukemic human bone marrow cells was recently set up in our laboratory. Attempts are made to study exogenous and endogenous (released from feeder layers of normal cells) factors which affect these processes. Attempts have been made here and in other laboratories to differentiate human leukemic blast cells with apparent success. The implications of this to understanding leukemogenesis and for potential therapeutic approaches are obvious. The mechanisms involved in the maturation process are under study.

5. Growth of leukemic myeloblasts in liquid suspension under the stimulus of a conditioned media factor produced by human embryonic culture cells.

6. Isolation and characterization of type-C virus which was produced by leukemic myeloblasts grown in liquid suspension and the development of biological and biochemical markers for human leukemia.

7. Markers: (a) an immuno-chemical technique for finding reverse transcriptase and other viral macromolecules in intact cells is now being developed. (b) Techniques for detecting viral specific nucleic acids in intact cells are also being developed.

8. Cell separation studies are being carried out to enrich subpopulation of leukemic cells which may contain the type C RNA tumor virus related markers and other biological markers.

9. Antibodies associated with the membranes of human myelogenous leukemia cells have been isolated. They are under study as to which protein antigens they interact with, i.e., are they leukemia cell specific, e.g., viral, etc.

Methods Employed

1. Human leukocytes were isolated and purified as previously described (J. Clin. Invest. 48: 105-116, 1969; Science 165: 400-402, 1969). PHA stimulation of purified lymphocytes has also been described (Biological Effects of Polynucleotides, Springer-Verlag, New York, 1971, pp. 303-334; Blood 37: 282-292, 1971).

2. DNA polymerase activities were purified and characterized as reported (Nature New Biology 240: 67-72, 1972; Proc. Natl. Acad. Sci. 69: 2879-2884, 1972; Proc. Natl. Acad. Sci. 69: 3228-3232, 1972; DNA Synthesis in vitro, Proceedings of the Second Annual Steenbock Symposium, 1972.

3. Viral reverse transcriptase was purified and studied as described (Nature 234: 194-198, 1971; J. Virol. 12: 431-439, 1973; Biochim. Biophys. Acta 454: 212-221, 1976).

4. Macromolecular synthesis, viability, mitosis in leukemic and normal cells, and the effects of specific agents were evaluated as described before (J. Natl. Cancer Inst. 46: 789-795, 1971; Science 165: 400-402, 1969).

5. In vitro leukopoiesis is studied by the soft agar technique developed by Paran and Sachs. In addition human myelogenous leukemic leukocytes are propagated in liquid suspension culture, as recently reported (Science 187: 350, 1975).

6. Induction of type-C virus from "non-producer" cells by iododeoxyuridine is carried out essentially as originally described by Rowe and colleagues. Infectious units focus formation and plaque assays for virus are carried out by conventional techniques.

7. Molecular hybridization studies are carried out by conventional and by newly evolved techniques. These include: (a) filter technique with DNA; (b) filter technique with RNA covalently attached (Proc. Natl. Acad. Sci. 70: 3219-3224, 1973); (c) Cesium sulfate gradient analyses; (d) S_1 nuclease treatment; (d) RNA-DNA hybridization by competition analyses (Methods in Cancer Research, Vol. XI).

8. Tissue culture, virus production, cell viability estimates, cloning of cells are all carried out by standard techniques. Established procedures for titering infectious, leukemic viruses (SX test) and transforming sarcoma viruses (focus formation) are routinely performed. Also, virus neutralization procedures are performed by standard procedures.

9. Virus quantitation, virus specific molecules, metabolism of viral RNA and proteins are studied by conventional techniques.

10. Cell separation studies are carried out using ficoll-hypaque gradients, sucrose density gradients and free flow electrophoresis (Lancet 1: 508-509, 1976).

Major Findings

1. Established in culture a continuously replicating leukocyte line from the blood of a patient with acute promyelocytic leukemia. After initial stimulation by conditioned media from a cultured human embryonic lung, this line is able to grow in the absence of conditioned media and shows continued evidence of myeloid differentiation.

2. One isolate of HL23 virus (derived from the leukocytes of a patient with acute myelogenous leukemia) has been shown to possess fibroblast transforming activity *in vitro*. This is an important observation because it could mark the isolation of transforming genes of human origin.

3. Isolation of conditioned media from PHA stimulated lymphocytes which promote the growth of T lymphocytes.

4. Detection of proviral DNA sequences related to the baboon endogenous virus and Rauscher murine leukemia virus in fresh tissues of some leukemic patients.

5. Demonstration that leukemic leukocytes synthesize one or more species of high molecular weight type C RNA tumor virus like RNA (50-70S), while bone marrow or blood leukocytes from normal donors do not.

6. A technique has been developed for the enrichment of type C viral sequences of high G+C content by banding of DNA in sodium iodide gradients. This technique will be useful in enriching of high G+C containing proviral sequences in human leukemic tissues for molecular hybridization studies.

7. A technique of competitive RNA hybridization has been developed for the detection of viral related RNA in leukemic tissues. Preliminary results indicate the presence of mammalian type C virus related RNA in tissues of some leukemic patients.

8. A technique of *in situ* hybridization of viral cDNA to RNA of fixed cells has been developed for the detection of type C RNA tumor virus RNA in cells producing virus. This technique will be extended to the detection of viral related RNA in human leukemic cells.

9. A new isolate of a primate type C virus has been obtained from tissues of a gibbon with acute malignant lymphoma and leukemia. This gibbon was obtained from Hall's Island, Bermuda. The new virus isolate is designated $GaLV_H$, and has biochemical properties similar to the other known isolates from gibbons.

10. Isolation of antibodies, from short term cultures of leukocytes from CML patients, that possess high neutralizing activity against feline and murine leukemia virus reverse transcriptases. Antibodies isolated from AML patients possess high activity against reverse transcriptase from HL-23 virus.

11. Purification of DNA polymerase gamma to homogeneity and preparation of antibody against this enzyme. This antibody will be useful in distinguishing viral reverse transcriptase and cellular DNA polymerase γ, since both of these enzymes can utilize $(dT)_{15}\cdot(A)_n$ as primer-template.

12. Development of a sensitive double antibody precipitation technique for the detection of cross reaction of antibodies with reverse transcriptase and DNA polymerase γ.

13. Demonstrated the presence of terminal deoxynucleotidyl transferase in CML patients in acute blast phase of the disease, its loss during treatment, and reappearance just before blast crisis. This enzyme is a potential biological marker for following these patients during treatment and remission.

14. Showed by cell separation studies that terminal deoxynucleotidyl transferase is present in a subpopulation of cells in normal bone marrow.

15. Demonstrated the association of reverse transcriptase with plasma membranes of virus transformed cells as well as from a patient with acute lymphocytic leukemia.

16. Demonstrated the presence of inhibitors on the plasma membranes from some leukemic patients that specifically inhibit simian sarcoma virus reverse transcriptase.

17. Optimized conditions for the preparation of large cDNA copies from primate type C viruses for restriction enzyme analysis, and for molecular hybridization studies.

18. Purified to near homogeneity the glycoprotein gp70 and p30 of simian sarcoma virus and raised antisera against p30. Preparation of antisera against the gp70 is in progress.

Significance to Biomedical Research and the Program of the Institute

As outlined in the Objectives, these studies are designed to obtain fundamental information on molecular and physiological control mechanisms and the pathogenesis of neoplasia with the ultimate goal of developing new and improved approaches for anti-tumor therapy. In addition, some studies are designed to develop biochemical "markers" of neoplastic cells.

Proposed Course

As described above, some projects will terminate and others will continue to be actively pursued.

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ANNUAL REPORT

DRUG EVALUATION BRANCH

DEVELOPMENTAL THERAPEUTICS PROGRAM

DIVISION OF CANCER TREATMENT

NATIONAL CANCER INSTITUTE

The Drug Evaluation Branch (DEB) mission is to develop and coordinate laboratory projects for the recognition, recommendation, and pre-clinical development of improved therapies against human cancer. This is achieved by (a) screening large numbers of synthetic and natural products for biological activities suggestive of clinical utility; (b) detailed testing of materials of interest reported from other testing programs and in published literature; (c) evaluation of "active" drugs for conditions of usage which may optimize efficacy - e.g., modifications of route and schedule of treatment, combination chemotherapy, and combined treatment modalities; (d) research aimed at devising improved methods for pre-clinical drug evaluation; and (e) developing the resources - e.g., animals, computerized data processing and retrieval, and ancillary services - required for conduct of the program.

Implementation is through contracts with qualified institutions. At the end of March, 1977, DEB directed 29 research or resource contracts and one PL-480 agreement directly related to drug evaluation at 20 institutions (16 in the U.S.A., four foreign) and funded at approximately $12,000,000 (Table 1). In addition, DEB monitored 31 contracts funded at approximately $7,500,000 for laboratory animal production and related genetic and disease control. An analysis of funding, research or research activities, and a brief summary of objectives for the contracts listed in Table 1 are appended to the report of the Director, DCT.

Addition of the DCT animal production resource contracts to DEB responsibilities was concomitant with the merging of the Drug Research and Development (DR&DP) and Experimental Therapeutics (ET) Programs to form the Developmental Therapeutics Program (DTP) and the transfer of the Mammalian Genetics and Animal Production Section (MG&APS) from the Office of the Associate Director to DEB. Currently, DEB includes the Office of the Chief (J.M. Venditti); the Automated Information Section (AIS) (J.F. Waters, Head); the Mammalian Genetics and Animal Production Section (MG&APS) (J. Mayo, Head); and the Screening Section (SS) (B.J. Abbott, Head). The Branch is staffed by 14 senior professionals, two junior professionals, and nine technical and clerical personnel. Major staff functions include (a) determination of scientific approaches to realization of mission objectives stated in the initial paragraph of this report; (b) devising appropriate experimental protocols; (c) implementation through contracts including generation of Project Plans, Contract Summaries, Requests for Proposal, review of proposals, and participation in Source Selection Groups; (d) serving as Project Officers for negotiated contracts and insuring scientific and technical sufficiency of performance; (e) assimilation, review, and summarization of data; (f) presentation of potentially improved therapies against human cancer to the Associate Director, DTP, the DCT Decision Network (DN) Group, and clinical groups; (g) presentation of major findings to the scientific community through publications; and (h) preparation of final reports relative to pre-clinical therapeutic efficacy of new drugs for inclusion in Investigational New Drug Applications (INDA) and clinical brochures. During the past 18 months, major DEB efforts were aimed at implementation of the new DCT screen for potential anticancer drugs (Chart 1) and increased collaboration with the Investigational Drug Branch in supplying new and updated reports for INDA's and clinical brochures.

The screening plan depicted in Chart 1 was facilitated by the successful development of animal-tumor systems presumed to be models of major human tumor classes and fundamental studies demonstrating the utility of human tumor xenografts to screening. Previous studies of correlations between drug activity in animal screens and efficacy against human cancer had highlighted mouse leukemia L1210 as the animal model which best discriminated between clinically active and inactive drugs. However, these retrospective studies could be reasonably criticized because of the abiding - and sometimes exclusive use of L1210 as a primary screen during the years when most clinically active drugs were selected. No prospective study of "animal-man" correlations had been attempted. The present antitumor animal screening program represents one arm of such a prospective study; the other being the eventual clinical trials of the drugs uncovered. In order to provide the required pre-clinical data, we began to emphasize during the previous reporting period, testing of new synthetic compounds and "active" isolates from natural products against transplantable colon, breast, and lung tumors of mice; human tumor xenografts of these tumor types grown in athymic mice; and mouse tumors of known value (e.g., L1210 and B16 melanoma). This decision impacted seriously on many aspects of screening as it had been practiced. The relatively greater effort required to test a material against most of the tumors in the spectrum - in contrast to the lesser effort to screen against L1210 - plus the increase in the number of models, limited the number of materials that could be screened in the tumor spectrum to 500-1,000 per year. Means of selecting these materials were needed. Having considered a number of alternatives, we determined that a reasonable and feasible plan would be to utilize, as an initial in vivo "pre-screen," a model known to be sensitive to most classes of clinically effective anticancer drugs, but sufficiently restrictive to avoid overloading the spectrum. L1210 was considered to be too restrictive for a pre-screen designed to minimize the probability of missing a material likely to show activity against one of the spectrum tumors. Comprehensive review of existing data pointed to mouse leukemia P388 as an appropriate pre-screen because its response to most useful drugs was qualitatively similar, but quantitatively greater than the response of L1210. In accord with the present plan, natural product isolates and synthetic compounds emerging from the P388 pre-screen are tested against the entire tumor spectrum. Despite the necessity of using a pre-screen for selection of agents for broader spectrum screening, we recognize that no single animal model is known to predict perfectly for clinical utility. Therefore, the input to the spectrum is augmented with materials of interest based on activities in other screening programs and selected biochemical and biological assays. Such a material is committed to spectrum testing regardless of its pre-screen performance. Crude natural products are pre-screened in vivo against P388 and also against KB cells in culture. When correlative activity is found the more time and cost efficient cell culture system is used as the primary bioassay tool for following activity through the fractionation procedure to isolate the active antitumor component. Most synthetic compounds and crude plant extracts enter the screen without previous biological data. In contrast, most fermentation products screened in vivo are selected from microbiological or biochemical assays or are products of microbial biotransformation of known active drugs. These materials, when purified, may be tested against the tumor spectrum regardless of their effects against P388. As materials emerging as active from any of the spectrum models are subjected to adequate clinical trial, this approach to screening will provide, prospectively, critical information relative to the value of the animal tumors used individually or in concert.

Status of Current DCT Screening Plan (Chart 1)

The change from L1210 to P388 as the in vivo pre-screen was completed. The erythroleukemia model, discussed in last year's report as a potential in vitro ancillary pre-screen for synthetic agents, was found to be not feasible for large scale screening. At present, synthetic compounds are not screened in vitro except when the amount received is insufficient for the P388 pre-screen or when such testing is specifically indicated on a rational basis. Of the mouse tumor types included in the DCT panel of antitumor screens, the leukemia L1210, B16 melanoma, Lewis lung carcinoma (LL), and colon 26 (C6) screens are fully implemented to accommodate the projected 500-1,000 compounds per year. Mouse colon tumor 38 (C8) was a routine part of the panel for much of this reporting period, but is now used selectively and for detailed evaluation of drugs with activity against mouse colon tumors. The CD8F1 mouse mammary carcinoma (CD) screen is expected to accommodate the screening of 250 compounds during the next year. The new screening plan reflects no change in the initial screening of natural products. The status of the human tumor xenograft screens is discussed separately below.

During CY 1976, screening was initiated on 33,059 materials as compared with 39,370 in CY 1975; the difference being a reflection of the decreased number of synthetics - 30,979 in 1975 and 21,941 in 1976. An additional 8,388 materials were screened for the first time during the first quarter of CY 1977. Of the 41,447 materials input during the 18 month period, January 1, 1976 through March 31, 1977, 27,402 (66%) were synthesized and 14,045 (34%) were crude natural products - 9,783 plant extracts (24%), 2,036 animal extracts (5%), and 2,226 fermentations (5%).

A total of 241,857 screening tests were performed in tumor bearing rodents in CY 1976 compared with 283,607 tests in CY 1975 (Table 2). During the first quarter of CY 1977, 56,697 such tests were done. An in vivo test is defined as a treated group of six to ten animals. The decrease in number of tests from 1975 to 1976 was a consequence of the planned decrease in synthetic input. Table 2 shows the testing level for each of the eight quarter years from April 1, 1975 through March 31, 1977. Major changes in the distribution of tests by test system in accord with the new screening plan can be seen. The decrease in L1210 testing concomitant with the increase in P388 testing for new (non-SAC) synthetics was a consequence of the deletion of L1210 as the primary screen and the institution of the P388 pre-screen. The consistent level of testing for Selected Agents (SAC) against L1210 and B16 reflects the continued use of these models for secondary screening and as part of the DCT tumor panel. The high testing level for SAC compounds against P388 resulted from the use of this model for detailed drug evaluation of active agents (e.g., route and schedule studies). There was a progressive increase in the use of the new mouse tumor models (C6, C8, CD) for drugs that passed the P388 pre-screen. The decreased use of Lewis lung carcinoma from October, 1976 through March, 1977 was due to the increased use of solid tumor screening capacity for the others. While the total testing level decreased from 1975 to 1976, the resources required to carry out the screening program have remained essentially constant over the two year span. This is attributable to a number of factors including (a) the increased effort in time and labor costs to conduct a test against one of the solid tumors (B16, LL, C6, C8, and CD) relative to the cost of an L1210 or P388 test, and (b) the need to utilize ten mice per test group for the solid tumors and six mice per test group for the P388 pre-screen in contrast to the three mice per test group for the L1210 "mini-screen" which was the initial screen during most of the latter three quarters of 1975 (Table 2). Program statistics reported herein were summarized from management reports generated by the Value Engineering Company (33706) which develops and maintains the DEB biological data file. The figures shown in Table 2 include all tests submitted to the automated data processing contractor. Since means for computer processing test data for newer test systems may have been devised only recently or are still being developed, the figures shown in Table 2 may be incomplete in some instances. Since solid tumor tests with active materials may require 60 to 90 days for completion, the figures for the first quarter of CY 1977 may not include all tests initiated.

Future Needs Relative to the DCT Tumor Panel

It has been suggested that a major determinant in the selection of a new "analog" may be a qualitatively different spectrum of antitumor activity. The originally projected 500-1,000 input of compounds into the panel excluded analogs of known active drugs; the latter being tested in a separate project by specific protocols related to the activities of the parent (Arthur D. Little, Inc., ADL, 53765). It is unlikely that all analogs could or should be subjected to panel screening, but we expect that a considerable number emerging from initial testing will be entered into the panel. These should be identified by the appropriate Analog Committee. Consequently, we can expect that the 500-1,000 panel input figure will be too low and a reasonable increase in capacity for panel screening will be needed. Our ultimate aim should be to have all panel systems available in all screening labs. In addition to providing the anticipated increased need for panel testing, the realization of this aim will greatly facilitate the overall evaluation of the drugs because we will be able to assign each drug to one lab for all mouse tumor testing (including follow-up testing and confirmation) and require a comprehensive final report from the contractor. At present, staff must schedule the individual experiments in various laboratories and then must integrate and summarize the results. Specifically, our short range plan would add the mouse colon tumor to the systems used at Hazelton Laboratories (57007), ADL (57006), WARF Institute (53770), and Mason Research Institute (MRI, 57003); it is now in use at the Southern Research Institute (SRI, 43756), IIT Research

Institute (IITRI, 57004), and Battelle Memorial Institute (BMI, 57005). The mouse mammary carcinoma, CD, is presently used at the Catholic Medical Center (CMC, 67081), IITRI (57004), and SRI (43756). Extension of CD testing to additional screening contractors is a goal for the next year. Full implementation of the CD8F1 model has been slower because, until recently, the only source of the animals was CMC. We are now breeding these hybrids elsewhere and the available supply should increase dramatically over the next year. However, we should plan to increase the capacity to breed CD8F1 mice. In addition, we should keep in mind that the CD8F1 model is presently used as the first generation transplant from the spontaneous tumor. The growth rates of the spontaneous tumors tend to be dissimilar, and these differences carry over to the first transplanted generation. To minimize differences in growth rate, IITRI is investigating the possibility of using several spontaneous tumors rather than a single tumor to prepare the inoculum for the transplant. In addition, we plan to have IITRI establish the CD tumor in continuous transplantation and compare growth rates and stability among various long transplanted lines and with first generation transplants.

It is very likely that the assessment of the value of the specific tumors comprising the panel will require five years since it will depend on adequate clinical evaluation of drugs showing activity in any animal model. Some panel tumors were selected with a minimum of information that could facilitate even a guess as to their usefulness. For example, C6 was selected from a number of available transplantable colon tumors, each with its own distinctive and attractive characteristics. Other mouse solid tumors are available. We would like to continue to explore the development of mouse lung tumor models (a recently completed project to judge the potential usefulness of the Nettesheim squamous cell carcinoma indicated that it has no advantage over LL). In order to provide basic information that would permit substitutions and additions to the panel if clinical information indicates that a current system is not prospectively useful, we should plan to continue development of alternative colon, mammary, and lung tumors and test a limited number of drugs in them. For example, all drugs found active against C6 should be tested in the three or four other mouse colon tumors that have been developed in this program. Similarly, drugs active against CD should be widely tested against a spectrum of rodent breast tumors because identification of an equally useful model devoid of the special problems associated with the present model would be very desirable. Drugs directed to the treatment of a particular tumor type on a rational basis should be tested against a spectrum of tumors representative of that type.

Given the limitations described above, the mouse tumors in the panel do represent, with regard to organ of origin, the major human tumor types. Nevertheless, there are others, in current limited use, which would be models of choice for the testing of certain rationally designed drugs. Such models, developed in this program, include animal brain tumors, hormone dependent and hormone responsive tumors (ovarian, testicular, prostatic, and breast), and a transitional cell carcinoma of the bladder. The number of drugs for which testing against such tumors is indicated has strained our capacity for such testing. In fact, the capacity is almost non-existent except for the brain tumors and such testing, when done because the indications for it seem overwhelming, often requires a very special effort in tooling up. Our capacity to test in these models should be increased. For example, the demonstrated activity of cis-Pt-II against a mouse ovarian tumor is certainly consistent with its clinical efficacy. The ability of this tumor to prospectively identify agents with activity against human ovarian carcinoma will never be known unless it is used. (Actually, the same consideration applies to human ovarian xenografts, at least one of which responds to Hexamethylmelamine.) We need to augment our capacity to utilize these ancillary models because (a) the greater dependence on intellectually based input of materials may suggest compounds for which such a tumor is the model of choice, (b) the increased presentation of new agents to DN-2A from independent programs may result in the development of drugs which cannot be studied further in our routine systems, and (c) output of compounds from the Drug Development CREG Program may require the use of diverse animal models. Although the CREG-RFA asks that the submitter conduct some initial testing, it also specifies that we will conduct whatever additional testing is needed. It is unlikely that many submitters will propose to do the comprehensive in vivo testing required to establish antitumor selectivity in a quantitative way.

Human Tumor Xenografts

Four contracts: the Stehlin Research Foundation (SRF, 67073); BMI (67099); MRI (57030, monitored by LCP); and the Chester Beatty Research Institute (CBRI, 43736); and R.K. Johnson's (LCP) intramural program comprise the DCT effort in this area. SRF is concerned chiefly with the development of models, studying their biological and operational characteristics, and recommending them as panel screens to be used on a large scale at BMI. The MRI contract is concerned with the development of new tumor models in general. In January, 1977, in order to facilitate maximum coordination of this entire area of research, DEB held a workshop including all concerned parties except CBRI. The workshop assessed the present state of the program and decisions were made regarding the immediate future. It was clear that while a number of models were being characterized, chemotherapy trials had been limited to well established drugs. The testing of 500-1,000 drugs per year remains a long-range goal, but we should be able to reach 750 compounds per year (or 250 compounds against each of three models) capacity within the next year. This will depend on the rate at which nude mouse production is scaled up. Program requested that we select three tumors - one breast, one colon, and one lung - for immediate inclusion in the panel; selection to be made on the degree of growth reproducibility over successive transplant generations. All participants felt that the selection of three tumors at this time would be detrimental to successful assessment of xenografts as models because there was no data to assure against selection of a tumor that would represent the exception to most human cancers of that type. However, those tumors which were felt to be relatively stable over a sufficient number of transplant generations were identified. It became apparent that the number was considerably less than we had originally envisioned. This was because of the lack of standardized nomenclature for the tumors. The practice of ad hoc nomenclature within each laboratory was common, and the decision was reached to standardize nomenclature throughout our program. Eight tumors were regarded as sufficiently well characterized to be panel candidates, and a tumor code which could be programmed into our computerized biological data file was assigned. These are listed in the following table.

Human Tumor Type	DCT Tumor Code	Previously Used Names	Lab of Origin	Other Labs Now Carrying Tumor
Colon	CX-1	HT-29	MRI	BMI, NCI
	CX-2	CA-1, CN-1	SRF	BMI, NCI
	CX-3	BE-1	SRF	BMI, NCI
	CX-4	H-25	SRF	--
Breast	MX-1	Clauser, BR-12	SRF	BMI, MRI
	MX-2	MDA-231	SRF	BMI, MRI
	MX-3	Ward	SRF	--
Lung	LX-1	Doyle	SRF	--

As is the case with the mouse tumors, each of these has its unique desirable characteristics. The need to make a further selection was clear. Even if we had not been asked to narrow the number in the panel to three, we could not effectively use a greater number within our present capacity and mouse supply. Moreover, the ultimate screening parameter of activity is regression of tumors or increased lifespan of animals treated when the tumors are well established and palpable at the site of implantation. Each test is expected to require 60-90 days. Thus, the need to reduce the number of models for chemotherapy trials of established tumors was clear. Currently, we are investigating a plan to limit the number of tumors used in "panel" testing on a scientific rather than arbitrary basis. MRI has demonstrated that at least two of the breast tumors and two of the colon tumors can be grown successfully (100% takes) and reproducibly with respect to growth rates when implanted under the kidney capsule of nude mice. Using a precise and simple protocol, the ability of a drug to reduce or impair growth of the tumor can be determined in 11 days. MRI has been able to screen an extraordinary number of standard drugs in this model in a relatively short time. The assessment of this pre-screen to select the appropriate model(s) for evaluation of a drug against established subcutaneous (SC) human tumor xenografts was recently begun. Nine drugs were selected from those in current DCT development to clinical trial. Using the eight tumors listed above, MRI will conduct testing in the kidney capsule model, SRF will test in the kidney capsule model and against the established SC tumor, and BMI will test against the established SC tumor. This study is aimed at establishing the kidney capsule model as a pre-screen for selection of the tumor to be used for the chemotherapy trial in the panel. The long-range plan is to test candidate drugs for the panel against a wide spectrum of human tumor kidney capsule xenografts. For each tumor type, the most sensitive tumor in the kidney capsule test will be the tumor used for the definitive panel testing against the advanced tumor. Additional related immediate plans call for the development of additional lung models. LX-1 would not have been chosen - the "take" rate is less than 80% - except that it is the only one in our program that has survived a substantial number of transplant generations. We have obtained the P246 human bronchial carcinoma responsive to Hexamethylmelamine from CBRI and we plan to add it to the BMI human tumor spectrum. We plan to survey the tumors used in other programs (e.g., Dr. Sato's group in California) in the search for non-oat cell tumors. We should establish lines of other human tumor types (e.g., ovarian) for use on a non-routine basis.

Major Findings

In screening against established SC human tumor xenografts, BMI (67099) reported that the mammary carcinoma, MX-1, regressed under treatment with Cyclophosphamide (CPA, NSC-26271), Melphalan (L-PAM, NSC-8806), or AT-125 (NSC-163501); 5-Fluorouracil (5-FU, NSC-19893), Methotrexate (MTX, NSC-740), MeCCNU (NSC-95441), BCNU (NSC-409962), PALA (NSC-224131), and the Anthraquinone (NSC-196473) retarded tumor growth; while AMSA (NSC-249992), Anguidine (NSC-141537), Bruceantin (NSC-165563), L-Alanosine (NSC-153353), Lymphosarcin (NSC-208642), Chlorozotocin (NSC-178248), and DL-Amygdalin (NSC-251222) were without effect. Against the human colon xenograft, CX-1, Bleomycin (NSC-125066) produced tumor regression while 5-FU, MeCCNU, and CCNU were inactive. Against colon xenograft, CX-2, MeCCNU produced regression; CPA, L-PAM, 5-FU, Lymphosarcin, and Mitomycin C (NSC-26980) retarded tumor growth; while 5-FUDR (NSC-27640), MTX, AMSA, Anguidine, Bruceantin, L-Alanosine, AT-125, Chlorozotocin, Hexamethylmelamine (HMM, NSC-13875), and Bleomycin were inactive.

SRF (67073) tested five human breast carcinoma xenografts for sensitivity to selected agents. Each tumor was shown to be composed of human cells by karyotyping and/or G6PD isozyme determination. Each gave a 90% or higher rate of positive takes in nude mice. Two tumors responded to Adriamycin (ADR, NSC-123127); three responded to 5-FU. Only one responded to both ADR and 5-FU, and the patient responded to the combination of these drugs. The SRF group continued to study the biological characteristics of human tumor xenografts to aid in the selection of xenografts for screening; e.g., stability over sucessive transplant generations, constancy of human chromosome pattern, growth rates, and responses to standard drugs. In the course of these studies, the responses of MX-1 to L-PAM, 5-FU, and ADR were confirmed. All of the breast carcinomas studied to date have exhibited a striking increase in growth rate with successive passages. However, growth rates have become stabilized for the three selected as potential screens (MX-1, 2, and 3). The work at MRI (57030) in developing the mouse kidney capsule xenograft pre-screen was discussed earlier and a more detailed report of activities under this contract will be found in the current report of the Laboratory of Chemical Pharmacology, Tumor Biology Section together with major findings from the intramural activities of that Section.

Xenograft studies conducted at CBRI (43736) utilized thymectomized and radiated mice rather than "nudes." However, these studies have been very productive from the program viewpoint; probably because they are part of a comprehensive drug developmental program at CBRI with increasing emphasis on biochemical pharmacology. A concerted effort to identify and isolate metabolites of HMM and to test them and derivatives against the human lung xenograft, P246, resulted in identification of two with activity equivalent to that of HMM, but which are substantially more soluble in water. This work represents an example of how studies directed to solving problems arising in the clinic may uncover new, potentially superior drugs. HMM has been shown to produce significant responses against human bronchiogenic carcinoma, but oral administration is necessary because of its insolubility. Resulting GI side effects limits its use. Recent attempts to develop an IV dosage form involved the use of gentisic acid complexes. IV injection into the rabbit ear showed that the gentisate was more irritating than HMM (Laboratory of Toxicology). The CBRI group studied the demethylation metabolites of HMM inasmuch as increased solubility is often a consequence of metabolism. A number of demethylated metabolites were found and derivatives of some were made. Comprehensive studies of solubility, stability, and activity against human xenograft P246, which responded well to HMM in contrast to most transplantable mouse tumors, identified Pentamethylmelamine hydrochloride (PMM, NSC-118742) and Trimethyl-triethylol-melamine (NSC-283162) as drugs worthy of further development. Both have passed DN-2A of the DCT Linear Array. At present we are investigating the antitumor activity of Trimethylmelamine (NSC-57552) which is reported to be a major HMM metabolite in man.

A total of 15 compounds passed DN-2A from January 1, 1976 through March 31, 1977. The two HMM analogs discussed above; two adenosine deaminase inhibitors, 2-deoxycoformycin(2-dCF, NSC-218321) and EHNA (NSC-263164) because they potentiated the activity of adenine arabinoside (Ara-A, NSC-404241) against P388 and L1210; Rachelmycin (NSC-219877), active against B16 and P388; NSC-240899, active against L1210; an isolate from Bouvardia Ternifolia (NSC-259968), active against B16; Dihydro-5-azacytidine (NSC-26488), active against L1210 and because of increased solubility and greater in vivo stability than 5-Azacytidine (NSC-102816); a steroid (NSC-282175) shown to be effective against a rat mammary tumor by the Worcester Foundation for Experimental Biology; NSC-141401, active against B16; Rubidazone (NSC-164011), a Daunomycin (NSC-82151) analog with equivalent anti-P388 activity and diminished cardiac toxicity relative to Adriamycin (ADR, NSC-123127); NSC-182986 because of activity against the mouse ependymoblastoma and IC-implanted L1210; and NSC-191959, a high molecular weight glycoprotein, active against B16 and P388. L-alanosine sodium salt (NSC-153353), active against L1210, was selected over the DL form; and Levamisole (NSC-177023) because it is presently in clinical trial as a potential enhancer of antitumor immune response.

During the past year DEB submitted animal therapeutic activity data for inclusion in INDA's and clinical brochures for 3-Deazauridine (NSC-126849), Rubidazone, Bruceantin, Chlorozotocin, and AMSA. To assist intramural scientists in the conduct of biochemical and pharmacological studies with drugs in development, the Office of the Chief, DEB responded to 20 requests of summarized data and ancillary information. In addition, AIS responded to 350 multiple-drug queries requiring specially formated computer output summaries; an increase of 80% over the previous year. As the repository for the DCT file of pre-clinical biological data, DEB has collaborated extensively with the DCT Analog Coordinating Groups by supplying required testing results.

The Platinum Coordinating Group was formed under the Charimanship of a DEB Senior Staff member. The group reviewed existing data on metal-containing complexes submitted over the past several years. Compounds were ranked based on activity, solubility, purity, availability, and structural considerations. Promising candidates are being procured and scheduled for further evaluation in a variety of animal solid tumor models (breast, ovarian, colon, bladder, etc.) and in a rat renal toxicity model developed by the Laboratory of Toxicology. Formal reports are submitted quarterly to the Division Director.

The Southern Research Institute (SRI, 43756) multifaceted contract continued to conduct (a) screening and detailed drug evaluation in accord with DEB generated protocols, (b) developmental studies with methodologies and approaches formulated and tested by the contractor, and (c) exploratory studies aimed at DCT objectives, but conceived by the contractor. Approximately 40 research tasks were carried out during the past year. Only a few significant findings can be mentioned in this report. For a comprehensive summary of findings, refer to the contractor's Annual Report of January 15, 1977, and publications (see "Contractor Publications" appended to this report).

The anti-P388 activity of Ara-A was markedly increased by combination treatment with the adenine deaminase inhibitors, 2-dCF and EHNA. Against L1210, Ara-A alone was inactive, but was very effective when combined with the enzyme inhibitors. Ara-A plus 2-dCF was active against first passaged AKR leukemia, IV implanted LL, and Ridgway osteogenic sarcoma (ROS). Limited, but consistent data failed to indicate that response of mouse tumors to Ara-A treatment is determined by intracellular adenine deaminase levels. Enzyme inhibition by 2-dCF was essentially irreversible while inhibition by EHNA was reversible.

An L1210 subline, resistant to CPA (L1210/CPA) retained full sensitivity to BCNU, CCNU, or Dianhydrogalactitol (DAG, NSC-132313) and partial sensitivity to L-PAM or the Piperazinedione (PD, NSC-135758). L1210/BCNU was cross-resistant to CCNU, MeCCNU, or BIC (NSC-82196), but retained sensitivity to CPA, L-PAM, DAG, or PD. L1210/L-PAM was cross-resistant to nitrosoureas and CPA, partially resistant to BIC, but remained sensitive to DAG or PD. While all of the drugs in this study have demonstrated alkylating functions, the various patterns of resistance and cross-resistance observed indicated that major sites of biochemical attack and/or mechanisms of DNA repair may differ significantly among them.

P388 sublines resistant to Vincristine (VCR, NSC-67574), ADR, or Actinomycin D (Act. D, NSC-3053) were cross-resistant to VCR. However, P388/VCR was cross-resistant to Act. D or VCR. None of these variants showed resistance to CPA or *cis*-Pt-II (NSC-119875). P388/VCR demonstrated collateral sensitivity to MTX or 5-FU.

In studies of the evaluation of single agents and combinations against mouse solid tumors, the most impressive antitumor activity against established C8 was found with Anguidine and 5-FU plus Anguidine. Anguidine alone was more effective than 5-FU alone, but was substantially inferior to the combination. Tests against a limited spectrum of mouse colon tumors showed the following additional single agents or combinations to be quite effective against one or more tumors: 5-Fluorodeoxyuridine (5-FUdR, NSC-27640), BCNU, MeCCNU, Homoharringtonine (NSC-141633), *cis*-Pt-II, DAG, PD, CPA, MeCCNU plus 5-FU, and 5-FU plus Cytosine arabinoside (Ara-C, NSC-63878).

First generation SC transplants of spontaneous mammary carcinomas of CD8F1 mice (CD) responded to 5-FU, VCR, ADR, L-PAM, and CPA; but not to MTX. These results confirmed the original findings at the Catholic Medical Center (67081) relative to the response of this tumor to drugs reported effective against human breast carcinoma. With the CD model, the SRI group observed marked variability in control tumor growth from experiment to experiment. This variability weakens the usefulness of this conceptually important model in terms of quantitating and confirming results. Analyses of tumor growth kinetics suggested a correlation between "late" tumor development and low cell viability of the tumor inoculum. The doubling times (Td) of spontaneous donor tumors varied considerably, but carried over to the first generation transplants although the latter tend to display Td's somewhat shorter than their respective spontaneous parent tumors. However, the cell cycle parameters (cell cycle times and cell cycle phase times) and Thymidine indeces were not very variable among individual spontaneous tumors and seemed to be characteristic of the tumor system. Thus, the variation in growth rates could not be attributed to differences in specific kinetic parameters. Our approach to increasing consistency of growth among first generation recipients was discussed earlier.

ADR, Act.D, CCNU, MeCCNU, CPA, 5-FU, Ara-C, or VCR was combined with whole body hyperthermia (WBH) in treating mice with one or more of the tumors, P388, LL, ROS, a mammary adenocarcinoma (MaCa 16/C), or C8. As employed in these studies, WBH failed to reproducibly potentiate drug activity in any instance. No antitumor activity was seen when mice with LL, B16, mammary adenocarcinoma, or L1210 were maintained continuously in hyperbaric hydrogen (97% H2 and 3% 02) at eight atmospheres for seven days.

The immunostimulant, BCG, was used in conjunction with surgery to treat mice with LL, ROS, or MaCa 16/C. BCG was also evaluated against LL in combination with surgery plus MeCCNU and against MaCa 16/C in combination with surgery plus ADR. In no instance did BCG enhance therapeutic efficacy. In contrast, the immunostimulant, C. Parvum, when given as a single IV dose to C3H mice bearing small, but palpable transplantable methylcholanthrene-induced fibrosarcomas, caused complete tumor regression following a period of further growth; a significant proportion of the mice were cured.

The results of extensive studies of surgery-chemotherapy adjuvant treatment of metastatic mammary and colon tumors established that if a drug or a combination of drugs was highly active against moderately advanced primary tumor masses, this treatment was also highly active postsurgery against residual metastatic disease.

Arthur D. Little (ADL, 53765) continued projects for analog screening, combination chemotherapy, and combined treatment modalities. During the past year, special protocols for testing compound classes of interest were modified in collaboration with the DCT Analog Coordinating Groups. Initial screening was carried out on 1,226 "analogs." Active analogs were reported to the appropriate Coordinating Group and further development of selected compounds was planned in collaboration with the group. Where no Coordinating Group exists for a structural class, further development is carried out in collaboration with the Screening Section, DEB, with advice of the Drug Evaluation Committee.

Sixty-four combination chemotherapy experiments were conducted against L1210, P388, B16, or LL. Instances of potential superiority over single drug treatment are listed in the current report of the Screening Section, DEB. In other combination chemotherapy studies, C. Parvum potentiated ADR activity against P388.

Over the past year, ADL (53765) completed ten studies to determine the effectiveness of systemic antitumor drug treatment plus localized radiation against mouse transplantable solid tumors (ROS or B16). The drugs used were CPA, ADR, MeCCNU, HMM, VCR, BCNU, and 5-FU. When radiation was given at an optimal dose (≥600 rads/day X 5), combined modality therapy was generally not superior to radiation alone. When radiation dosage was reduced to a palliative level (<600 rads/day X 5), the combined modality was generally superior to either arm alone.

In vivo systems were developed to examine the effectiveness of radiation sensitizers, agents designed to sensitize hypoxic tissues to radiation and which are usually without antitumor activity when used alone. Metronidazole (NSC-69587) or RO-07-0582 (NSC-261037) was combined with whole body radiation against P388. Mice bearing ascites tumors were drug treated 30 minutes prior to radiation. Thirty minutes after radiation tumor cells from these donor mice were implanted into recipients in various dilutions. Calculation of the number of cells required to 50% tumor growth in the recipients showed that both NSC-69587 or NSC-261037 plus radiation produced greater cell kill than radiation alone. NSC-261037 was more effective than NSC-69587. The "delay in re-growth" of solid tumors; e.g., EMT-6 or B16, is being developed as a model to test the radiosensitization potential of chemical agents.

The Catholic Medical Center (60781) group reported that excess Thymidine augments the antitumor effect of 5-FU against CD and C6. In collaborative studies with Dr. Sol Spiegelman (Columbia University), there was a marked increase in the incorporation of 5-FU into mammary tumor RNA in the presence of excess Thymidine. It is postulated that in the presence of Thymidine, the main cytotoxic action of 5-FU is due to its incorporation into RNA.

Budgetary constrictions due to attempts to restrict escalation of expenditures in the face of progressive inflation have necessitated the termination of some research contracts. The Baylor College of Medicine (57018) project to evaluate the mammary hyperplastic alveolar nodule as a potential antitumor screen will be completed this year. Further use of the system in this program will depend on its demonstrated predictive value when final results are evaluated. The Yale University (53824) project to prepare and screen sugar analogs which might retard tumor growth by altering cell membranes, and the Yale University (67068) project to design, prepare, and test new agents as intercalators of DNA have made significant progress. However, both seem more suited to funding under CREG and will be terminated as contracts. These contractors have been encouraged to pursue the CREG mechanism.

Yale University (67068) project to screen antifols for inhibition of dihydrofolate reductase (DHFR) and to conduct biochemical and pharmacological studies, including clinical, on those with highest activity, is continuing. During the past year, nine compounds were screened against human leukemia DHFR, with two (NSC-282189 and NSC-275631) being evaluated further. The Baker antifol (NSC-139105) was found to accumulate in human AML cells to a greater extent than MTX as extracellular concentrations increase. Carboxypeptidase G1 and Baker's antifol acted synergistically against animal tumors. The quinazoline (TMQ, NSC-249008) was ten times more potent than MTX in inhibiting DNA synthesis in human leukemia cells, intracellular TMQ levels after one hour incubation were 10-100 times those of MTX. TMQ was more effective than MTX against L1210.

The Applied Mathematics and Data Analysis Project (ADL, 33727) continued to utilize screening data from all appropriate contractors to define criteria for activity and toxicity in recently instituted animal screens, analyze variance among screeners, and to analyze the influence of experimental variables (host strain, number of mice per test and control groups, treatment schedules, etc.) which modify the reliability of results. In addition, the contractor, using continuously updated animal and clinical data, has applied multivariate statistical methods to develop discriminant functions or mathematical models that yield the "best" prediction of presumptive clinical activity against each of four human tumors. Discriminant functions based on existing screening results in five animal tumor systems and available clinical data show that, when the animal systems are used in concert, the probabilities of predicting a false negative or false positive, respectively, are 5.4% and 20.3% for human breast carcinoma; 9.5% and 14.7% for colon tumor; 23.9% and 28.1% for lung tumor; and 15.9% and 30.5% for melanoma. The proportion of known clinically active compounds correctly classified by the mathematical models were colon (94%), breast (87%), lung (76%), and melanoma (67%). Refinement of this approach to assessing the relative contribution of each of a set of animal systems to successful selection for the clinic, will facilitate judgments regarding relative usefulness among our new animal tumor screens. Specifically, the objectives of this study of "animal-man" correlations are to determine (a) the human tumor(s) most likely to respond to new active drugs, (b) human tumors which are likely responders to older drugs which have not been adequately evaluated against them, (c) animal tumor models which contribute little to the prediction formula for a specific human tumor, and (d) animal models which closely simulate each other. We envision that the further development and use of such analytical procedures will result not only in improved clinical therapy, but also in a more efficient use of clinical and laboratory resources. While no single experimental model, _in vivo_ or otherwise, predicts perfectly and absolutely for activity against human cancer, we recognize that no alternative to the use of non-human experimental systems exists. Therefore, it is incumbent on us to select models that provide the best tools for realization of our objective. Screening is a dynamic undertaking, the models used in this program have changed considerably over the years, and we expect that they will continue to improve progressively.

Table 1

Contracts Monitored by DEB During FY 1977

Contract NO1-CM-	Institute
	Office of the Chief
33727	Arthur D. Little, Inc.
53765	Arthur D. Little, Inc.
57018	Baylor College of Medicine
67052	Biotech Research Laboratories
67081	Catholic Medical Center of Brooklyn and Queens, Inc.
43736	Chester Beatty Research Institute
43756	Southern Research Institute
67073	Stehlin Foundation for Cancer Research
53824	Yale University
57029	Yale University
67068	Yale University
	Automated Information Section
33706	Value Engineering Company
67086	Value Engineering Company
53821	Value Engineering Company
	Mammalian Genetics and Animal Production Section
	Thirty-one contracts for maintenance of genetic integrity of breeder animals, rodent production, beagle production, animal disease diagnosis, animal-tumor histocompatibility, and monitoring of laboratory animal handling practices.
	Screening Section
57006	Arthur D. Little, Inc.
67078	Arthur D. Little, Inc.
57005	Battelle Memorial Institute
67099	Battelle Memorial Institute
57007	Hazelton Laboratories
43755	IIT Research Institute
57004	IIT Research Institute
63832	IIT Research Institute
57040	Institut Jules Bordet
33720	Mario Negri Institute of Pharmacological Research

Table 1, Continued

Contract N01-CM-	Institute
57003	Mason Research Institute
67077	Miami, University of
67075	Southwest Foundation for Research and Education
53770	WARF Institute
67076	Wisconsin, University of
48002	Indian Cancer Research Institute (PL-480)

Table 2

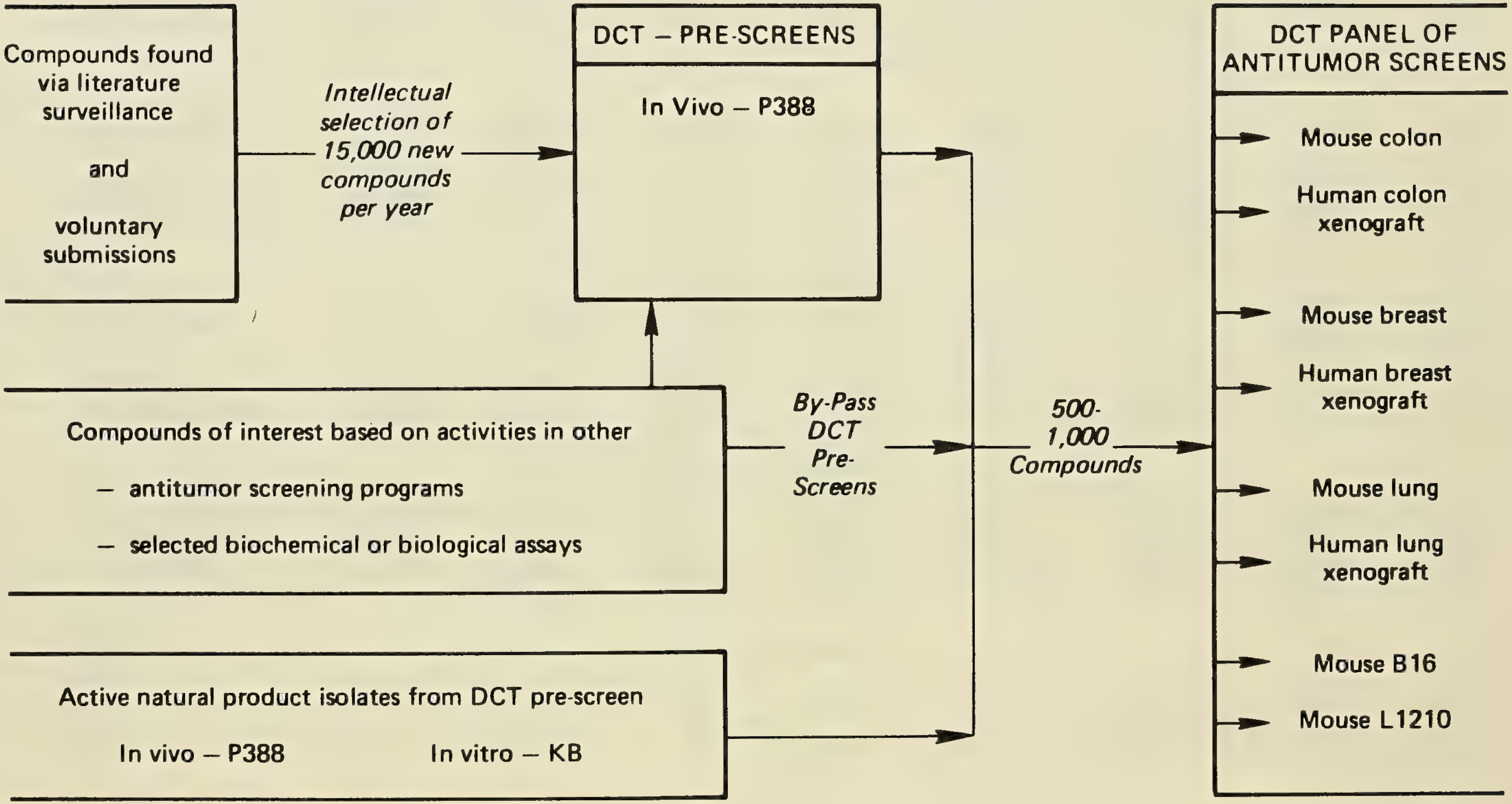
Distribution of In Vivo Screening Tests By Quarter (April 1, 1975 - March 31, 1977)

From	4/75	7/75	10/75	1/76	4/76	7/76	10/76	1/77
Through	6/75	9/75	12/75	3/76	6/76	9/76	12/76	3/77
Selected Agents (SAC)								
Leukemia P388	3,532	2,600	3,437	4,376	5,256	3,694	3,786	2,192
Leukemia L1210	3,440	2,610	2,445	1,747	1,970	1,542	1,115	681
B16 Melanoma	1,381	1,494	1,565	1,696	894	1,338	1,770	1,213
Lewis lung Ca	1,053	1,081	658	538	985	704	137	295
Colon 26	0	0	64	16	235	938	1,248	700
Colon 38	0	0	85	82	36	384	665	1,048
CD8F1 Ma Ca	0	0	9	33	107	81	200	330
Others	1,388	1,057	1,752	1,687	920	2,870	2,000	1,198
Sub-total	10,785	8,842	10,015	10,175	10,403	11,551	10,921	7,657
Non-SAC Synthetics								
Leukemia P388	2,026	2,338	9,071	20,619	18,694	24,816	23,121	25,819
Leukemia L1210	31,055	35,474	22,777	3,176	1,803	1,215	936	1,038
B16 Melanoma	420	650	215	209	133	334	132	277
Lewis lung Ca	18	136	188	258	65	91	145	103
Colon 26	0	0	0	0	6	0	25	26
Colon 38	0	0	4	4	0	0	28	35
CD8F1 Ma Ca	4	0	5	0	13	15	19	6
Others	224	154	250	290	177	128	108	156
Sub-total	33,747	38,752	32,510	24,556	20,891	26,599	24,514	27,460

Table 2, Continued

From	4/75	7/75	10/75	1/76	4/76	7/76	10/76	1/77
Through	6/75	9/75	12/76	3/76	6/76	9/76	12/76	3/77
Crude Natural Products								
Leukemia P388	9,958	9,852	15,363	17,646	15,386	16,998	12,264	15,601
Others	876	649	479	208	144	379	373	82
Sub-total	10,861	10,501	15,842	17,854	15,530	17,377	12,637	15,683
Natural Product Fractions								
Leukemia P388	17,327	19,470	12,806	11,371	8,913	9,831	8,472	5,866
Others	89	73	90	111	53	55	43	31
Sub-total	17,416	18,543	12,896	11,482	8,966	9,886	8,515	5,897
TOTAL TESTS	72,809	76,638	71,263	64,067	55,790	65,413	56,587	56,697

CHART 1 FLOW OF DRUGS THROUGH DCT SCREENS


Compounds found via literature surveillance
and
voluntary submissions
Intellectual selection of 15,000 new compounds per year
DCT – PRE-SCREENS
In Vivo – P388
Compounds of interest based on activities in other
– antitumor screening programs
– selected biochemical or biological assays
By-Pass DCT Pre-Screens
Active natural product isolates from DCT pre-screen
In vivo – P388
In vitro – KB
500-1,000 Compounds
DCT PANEL OF ANTITUMOR SCREENS
Mouse colon
Human colon xenograft
Mouse breast
Human breast xenograft
Mouse lung
Human lung xenograft
Mouse B16
Mouse L1210

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AUTOMATED INFORMATION SECTION

DRUG EVALUATION BRANCH

DEVELOPMENTAL THERAPEUTICS PROGRAM

DIVISION OF CANCER TREATMENT

NATIONAL CANCER INSTITUTE

The Automated Information Section (AIS) is responsible for the implementation and maintenance of the biological data processing system for the Drug Evaluation Branch (DEB), thereby providing for the automated processing, storage, and retrieval of biological data generated in testing laboratories under contract to the DEB, Developmental Therapeutics Program (DTP). Synthetic and natural products are tested in animals for antitumor activity and in cell cultures at various screening laboratories subsequent to entry into the system. By means of the biological activity or inactivity reported, decisions are made concerning the direction of compound acquisition, synthesis, fractionation of active crude natural products, and the recommendation of new agents for development toward clinical trial. The biological data base is capable of being queried by special programs thus providing special summary data for: 1) evaluation of the existing screens; 2) design and evaluation of new test systems; 3) review of compounds being considered for Division of Cancer Treatment (DCT) Decision Point 2A; 4) further evaluation of individual compounds and drug combinations; and 5) statistical information required for effective program management.

The AIS is staffed by three professionals, one computer aid, one secretary and has one vacancy which will be filled at the end of this report period. Professional staff serves as Project Officers and has responsibility for three contracts, all with the Value Engineering Co., NO1-CM3-3706, which is to be terminated in August 1977, NO1-CM5-3821, which maintains the Drug Distribution and Inventory System of the Division of Cancer Treatment, and NO1-CM6-7086, which provides the major service for the Biological Data Processing System. Contract NO1-CM3-3706 deals with a total modification of the Plant Header File and the Terminal Handler System design and implementation. The Plant Header File will be operational this Spring. In the first quarter of 1977 the Terminal Handler System was implemented in the seven large *in vivo* screening laboratories in the U.S. The installation of the Terminal Handler was received warmly by the new users, since many previous time consuming steps were eliminated. Also eliminated were many points of error including the keypunching of the data. This also lead to a decrease in the time to computer process the data thus cutting down on delays for decisions concerning active compounds.

The Combination Chemotherapy Report System became operational in Spring 1977 while the Solid Tumor Report System became operational in the Summer. These two reports are now becoming much more significant with the new test systems becoming more important. Computer validation for six new tumor systems and thirteen test systems was accomplished during the report period.

The latter half of the report period saw a revision of the Screening Data Summary introduced as a mock-up. Proposed changes to it include: fewer cryptic messages, addition of mortality distribution and deletion of coding for such things as tumored survivors. In conjunction with the already implemented Terminal Handler System and the proposed revised Screening Data Summary, the master files for the Biological Data Processing System will be totally modified to minimize computer expenditures and maximize usability.

The report period saw 350 special reports (queries) answered by the AIS. This is an increase of 80% for the previous comparable period and seriously increased computer rental charges by DCRT.

The AIS staff, in conjunction with the IITRI Screening Services Contract, NO1-CM4-3755, established two new data bases concerned with word processing; 1) Minutes of the Operating Committee and 2) Transactions of Compounds of Interest. Both of these tasks were significant in scope and undertaking. With these two management reports information will be more readily available to members of the DCT and DTP staffs.

The AIS staff by means of a new type of cathode ray terminal have developed a methodology by which data to be entered to several data bases are entered "off-line" and then transmitted to the DCRT computer. This is accomplished by recording the data on cassettes and then transmitted. In addition the AIS staff also utilize these cassettes for storing data, and for writing computer programs. This decreases the amount of "on-line" computer time utilized.

AIS and its Data Processing Contractor, Value Engineering Company, are presently collaborating with other DTP units in studies of means to interface the Chemical Information System and the Biological Data Processing System in order to facilitate structure/function relationships.

Table 1 provides the statistics for the report period and the trends of the DCT Animal Tumor Panel, use of the P388 Lymphocytic Leukemia, and the fewer number of random compounds screened are demonstrated. This shift is due to the change in screening practice and the initiation of screening in the Tumor Panel.

The Clinical Drug Distribution and Inventory System was expanded. All management reports were produced on schedule. In addition 81 special reports were produced. To meet the needs of the Investigational Drug Branch and the Pharmaceutical Resources Branch, principal users of these reports, it was necessary to increase their number and complexity. During this report period a significant number of clinical protocol data have been input to a special file. This is necessary to enable that investigators receiving clinical drugs are authorized. Verification is done via the computer and as a result fewer manual checks need be made. Also instituted during this period was a methodology for notifying protocol monitors that patient reports are received or overdue.

DCT PROGRAM STATISTICS

	1st Quarter 1977	Calendar Year 1976	Calendar Year 1975	Calendar Year 1974
1. Materials Added to the Master Files*				
Synthetic Compounds	6,173	21,127	26,511	29,946
Selected Agents	-------	383	175	107
Plants	2,225	6,448	6,393	5,375
Fermentation Products	642	1,876	7,328	718
Animal Products	172	1,365	2,086	1,675
Total	9,212	31,199	36,493	37,821
2. Tests Performed[+]				
L1210 Lymphoid Leukemia	3,704	38,669	160,089	211,832
P388 Lymphocytic Leukemia	65,340	245,949	125,958	124,396
Walker Carcinoma	183	648	1,318	909
B16 Melanoma	2,487	7,349	8,837	11,724
Lewis Lung Carcinoma	648	3,536	5,847	2,770
AK Leukemia	-------	50	462	925
Other Tumors	3,128	9,952	3,723	2,821
Total *In Vivo* (Excluding Toxicity)	75,490	306,153	306,234	355,377
Toxicity	745	4,683	5,554	9,215
Cell Culture	13,304	33,734	32,931	35,371
Alkaloid	245	400	350	500
Biochemical Assay	-------	24	28	-------
Total Non *In Vivo* (Including Toxicity)	14,784	38,841	38,863	45,086
Total of ALL Tests	89,784	344,994	345,097	400,463

* These figures are derived from the Developmental Therapeutics Program Biological Data Processing System and differ from those generated by the Drug Synthesis and Chemistry Branch which logs in materials when NSC numbers are assigned. A material first enters the data processing system when its receipt is acknowledged by the screener, which may be two to four months later.

[+] The marked increase in "Other Tumors" represents in part DCT Animal Tumor Panel testing which is delineated further in the current report of the Screening Section, DEB.

MAMMALIAN GENETICS AND ANIMAL PRODUCTION SECTION

INTRODUCTION

The primary function of the Mammalian Genetics and Animal Production Section is to provide healthy laboratory animals with properly defined genetic characteristics to various research investigators as follows:

1. DR&D Screening Program

2. Other NCI Research Contracts

3. Research Grants (strains not available commerically and surplus animals which are offered when available).

4. Veterans Administration Research Facilities (surplus animals which are offered when available).

5. NIH Intramural Program

A secondary function of this section is to coordinate the movement of transplantable experimental animal tumors from tumor bank sources into scientific laboratories.

The structure of the animal production program included:

4 Primary Genetic Centers

4 Genetic Production Centers

6 Rodent Production Centers

10 Hybrid Production Contracts

3 Beagle Production Contracts

4 Animal Disease Diagnostic Contracts

2 Histocompatibility Monitoring Contracts

2 Contracts with the National Academy of Sciences

The Section staffing consists of one senior staff member, on professional associate, one animal contracts assistant, and one clerk-typist.

The overall production of these facilites supplied approximately 6,000,000 laboratory animals during the calendar year 1976.

MICE

Inbred Weanlings	960,590
Inbred Pregnants	2,158
Hybrid Weanlings	4,309,192
Hybrid Pregnants	150
Outbred Mice	42,400
Outbred Pregnants	25

RATS

Inbred Weanlings	78,152
Inbred Pregnants	47
Outbred Weanlings	19,820
Outbred Pregnants	11

HAMSTERS

Weanlings	18,901
Pregnants	1,487

BEAGLES

912

The various animal supply contracts are structured as indicated:

PRIMARY GENETIC CENTERS

These centers serve as a breeding nucleus for the animal program. In order to insure that maximum quality control is maintained at this level, it is required that any animals received by such centers are derived into a germ-free state; i.e., Caesarian sections are performed under isolator conditions. These animals are then maintained as foundation colonies in defined flora isolators. These foundation isolators serve as a source of breeders for the larger expansion colonies which are housed under barrier conditions.

GENETIC PRODUCTION CENTERS

Foundation animals for these centers are received from isolator colonies via germfree shippers and are placed directly into the barrier-maintained facilites for propagation. Otherwise, the same quality control standards are maintained as described for the primary centers.

RODENT PRODUCTION CENTERS

The purpose of these contracts is to accomplish large-scale production of pure strains of inbred mice which will be supplied to fixed-price contractors for hybrid mouse production. The rodent production contract receives breeding stock from the genetic center expansion colonies. These breeders, therefore represent direct offspring of brother x sister matings. This production also takes place under rigid barrier-controlled conditions.

HYBRID MOUSE PRODUCTION CONTRACTS (FIXED PRICE)

These contractors supply the large volume of first generation hybrid mice for the screening program. Breeding animals for these colonies are received from the rodent production contracts. Animals are propagated under semi-barrier conditions.

BEAGLE PRODUCTION CONTRACTS

These animals are procured on a fixed-price contract basis from a qualified breeder and are primarily to the toxicology program.

DIAGNOSTIC CONTRACTS

These are service contracts which are utilized to monitor and upgrade the quality of animals moving within the animal production program and to the laboratory. Presently, they consist of one virus diagnostic service, one pathology service, one parasite diagnostic service, and one bacterial diagnostic service (primarily Salmonella and Pseudomonas species). A pathology services contract was awarded to Dr. Russell Lindsey of the University of Alabama.

HISTOCOMPATIBILITY STUDY CONTRACTS

These contractors perform genetic monitoring of inbred mouse and rat strains by means of skin grafts.

NATIONAL ACADEMY OF SCIENCES

A small amount of support via Task Orders 6 and 16 is supplied to the National Academy of Sciences. This support involves development and publication of standards and guidelines in the field of animal care and breeding. In addition, support is extended for holding conferences and meetings pertaining to the field of laboratory animal care.

DISCUSSION:

The Mammalian Genetics and Animal Production Section is utilizing the Small Animal Section of the Veterinary Resources Branch as a repository for inbred rodent sublines of NIH origin. Pedigreed litters have been supplied to our genetic centers from this section and will continue to be supplied on a regular and continuing basis. It is felt that in this manner we can reach and maintain a degree of genetic uniformity with our inbred and hybrid strains that was not possible previously when each genetic center was propagagted entirely from its own breeding stock which in effect meant that each center developed its own subline with consequent genetic drift.

In cooperation of Dr. Carl Hansen of the Veterinary Resources Branch we are searching for a more sensitive method of testing for histocompatibility with both the reference mice (NIH) and within our own colonies. In the interim we will continue reciprocal skin grafting using the NIH colonies as reference material.

We feel that progress is also being made in our Animal Health Program. Primary genetic centers have been "closed" to the extent that no animals enter except through Caesarian derivation. This policy has been maintained for approximately two years during which time foundation colonies with several inbred strains have been established by brother x sister matings within isolators. Larger expansion colonies are being established from isolator animals with defined flora. These animals are maintained under strict barrier conditions. Super-clean animals are now becoming available on a limited basis and are being supplied to selected rodent production centers. We anticipate that these centers will in the not too distant future be able to supply large numbers of clean healthy inbred strains for hybrid production and for laboratory usage.

All laboratory animals are monitored for viral, bacterial and parasitic diseases. We awarded a pathology service contract which will be very important to us in determining causative organisms when disease outbreaks occur in the laboratory or in the mouse colony.

SCREENING SECTION

DRUG EVALUATION BRANCH

DEVELOPMENTAL THERAPEUTICS PROGRAM

DIVISION OF CANCER TREATMENT

NATIONAL CANCER INSTITUTE

The Screening Section has as its principal objectives the selection and recommendation of new drugs as candidates for development toward clinical trial against cancer and the investigation of means for increasing the therapeutic usefulness of known antitumor drugs. Objectives are attained by (a) screening a large number of synthetic and natural products in vivo, and to a limited degree, in vitro; (b) conduct of biological assays for directing fractionation of natural products and isolation of active components; (c) in-depth evaluation of materials which emerge from primary screens with emphasis on the influence of factors such as route and treatment schedule that affect therapeutic efficacy; (d) analysis, evaluation, and presentation of pertinent experimental results to Division of Cancer Treatment (DCT) Staff; and (e) methodology research leading to development of improved assays for drug evaluation. Program is implemented through establishment and coordination of work conducted by contractual arrangement with qualified institutions, and through collaborations with DCT intramural laboratories. Activity against experimental tumors in vivo is the principal basis for recommending candidate drugs for development toward clinical trial and for studying optimal conditions for drug usage. In vitro activity against mammalian tumor cells or microorganisms provides a means of recognizing biological activity among synthetic agents submitted in insufficient quantity for in vivo screening, for following fractionation and isolation of active components of crude natural products with correlative in vivo activity, and for providing information relative to mechanisms of drug actions.

I. Staff Functions

During this report period the staffing of the Screening Section has included six senior professionals, one junior professional, and five technical and clerical personnel. Principal investigators are: B. J. Abbott (Head); M. C. Baratta (CO, retired January 1, 1977); R. I. Geran, N. H. Greenberg; M. M. Macdonald; and N. R. Melnick.

Staff serve as Project Officers for nine contracts devoted predominantly to in vivo screening; Arthur D. Little, Inc. (N01-CM5-7006), Battelle Columbus Laboratories (N01-CM5-7005 and N01-CM6-7099), Hazleton Laboratories (N01-CM5-7007), IIT Research Institute (N01-CM5-7004), Institute Jules Bordet (N01-CM5-7040), "Mario Negri" Institute for Pharmacological Research (N01-CM3-3720), Mason Research Institute (N01-CM5-7003), and WARF Institute, Inc. (N01-CM5-3770). Although the number of contractors conducting predominantly in vivo screening increased at the time of the last competition, the average test capacity per contract as well as total in vivo test capacity has been reduced due to cost inflation and program changes. Staff also serve as Project Officers for five in vitro screening contracts: Arthur D. Little, Inc. (NO1-CM6-7078), IIT Research Institute (NO1-CM6-3832), Southwest Foundation for Research and Education (NO1-CM6-7075), University of Miami (NO1-CM6-7077), and University of Wisconsin (NO1-CM6-7076). The Screening Section also collaborates with the Chief, Drug Evaluation Branch (DEB); Chief, Natural Products Branch (NPB); Assistant Director for International Treatment Research (DCT); and the Associate Director, Developmental Therapeutics Program (DTP) to monitor screening, detailed drug evaluation and methodology tasks performed under contracts with Catholic Medical Center (NOL-CM6-7081), Southern Research Institute (NO1-CM4-3756), Arthur D. Little, Inc. (NO1-CM3-3727 and NO1-CM5-3765), Upjohn Company (NO1-CM7-7100), Michigan Dept. of Health (NO1-CM3-3702), Institute Jules Bordet (NO1-CM5-3840), Japanese Foundation for Cancer Research (NO1-CM2-2054), The Chester Beatty Research Institute (NO1-CM4-3736), and Research Triangle Institute (NO1-CM9-2019). A Screening Services Contract with IIT Research Institute (NO1-CM4-3755) assists staff in the management of screening data which has become increasingly complex as protocols have been modified and expanded in recent years.

By reviewing biological effects of natural product fractions, the Screening Section establishes directions of effort under additional DTP contracts for natural product fractionation and isolation. Staff monitor the work of contract laboratories to insure that tasks are carried out as scheduled and with a high degree of scientific and technical proficiency and that projects are directed to the overall goals of DTP, DCT, NCI, and the National Cancer Plan. The staff select and design assays for uncovering new active drugs, design experimental protocols for contractor use, review results, recommend candidate drugs for clinical trial, and prepare data for publication and for review by the Drug Evaluation Committee, Operating Committee, Laboratory of Toxicology, Clinical Trials Area, and for DCT Decision Network review.

II. Significant Accomplishments

A. Determination of Antitumor Activity of New Agents

Primary screening in vivo consists of the initial testing of synthetic and natural products submitted to DTP for activity against transplantable mouse tumors. Screening protocols are designed to uncover a modest number of materials which are then subjected to progressively exacting requirements designed to select those with the greatest potential.

The DCT placed increased emphasis on the testing of fewer materials against a larger spectrum of animal tumors presumed to be models of specific human tumors. A program was initiated to utilize leukemia P388 in mice as a pre-screen to aid in the selection of agents for broader spectrum screening. P388 was selected because its response to most clinically effective drugs is qualitatively similar to that of L1210 but quantitatively more sensitive than L1210 and because it responds to most classes of clinically active antitumor drugs.

In addition to materials which show activity in our pre-screen, materials which have demonstrated interesting activity outside our program (e.g., antitumor activity in other screens, and other pertinent biochemical or biological activities) or because of structural considerations, are also tested against a panel of secondary in vivo screens. The current composition of the secondary screening panel includes mouse colon, breast, and lung tumors and human tumor xenografts of these types grown in athymic mice. However, it may undergo changes as new information becomes available. Other tumors such as brain, renal, ovarian or bladder may be used on a selective basis or as special interests warrant. Tumor systems are phased into the panel as permitted by the completion of developmental work, the availability of laboratory capacity, and in the case of human tumor xenografts, the availability of athymic (nude) mice. The panel now includes the mouse tumor systems, leukemia L1210, B16 melanoma, Lewis Lung carcinoma, Colon 26 and the $CD8F_1$ mammary tumor. The effort required to adequately test a compound in the panel of secondary screens is much greater than that required for follow-up testing of active materials in the past. Therefore, the number of materials tested in this panel will be limited (probably not more than 500-1000 per year), and this, in turn, has required a considerable reduction in the input of new materials to the pre-screen.

Materials regarded as potential candidates for clinical trial are studied for route and schedule dependency. Analogs of compounds already in development are compared with the parent compound in test systems selected on the basis of the biological activity of the parent. Crude natural products continue to be tested both in vitro (KB) and against the P388 in mice. Where activity is observed in both, the less expensive cell culture assay is used for bioassay to follow activity during the process of fractionation.

Tables 1-3 show the number of screening tests initiated and computer processed during calendar year 1976 and the first quarter of 1977. An in vivo test is defined as a treated group of from three to ten animals, and an in vitro test consists of a concentration - response assay from which the cytotoxic ED50 is extrapolated. Of 337,527 total tests for this 15 month period, 298,554 were in vivo (Table 2), and 38,903 were in vitro (Table 3). Tables 1-3 list the number of tests conducted with "selected agents" (SAC) which are chosen for further study because of activity in initial screening or other biological activity of interest, other synthetic compounds (Regular), structural congeners of known active compounds (Analogs), crude plant extracts, crude fermentation beers, crude animal extracts, and natural product fractions. These numbers include tests processed in our main computerized single drug data file. They exclude special testing such as combination chemotherapy and a separate solid tumor data file, and testing in new systems under current development for which results are not computer processed. Table 4 shows, by type, the number of materials first tested in any test system during the calendar year 1976 and the first quarter of 1977.

It also gives an analysis of the average lag time between the date a test is initiated and the date the test results are computer processed. The total, 41,477, is the number of materials for which testing was initiated during this report period and processed by May 2, 1977. The average lag time is greatest for selected agents and markedly lower for other synthetics or natural products. In general, primary screening is completed in a relatively short time. As materials are determined to be active or require specialized testing they are moved to the SAC file. The longer lag time for the latter is a reflection of both activity and more sophisticated test systems requiring more time for completion.

Table 5 shows, by test system, the number of tests carried out during this report period in the tumor test systems in the DCT panel of tumors. These numbers include the testing of compounds recommended for the complete panel as well as the testing of compounds recommended for a limited spectrum of panel tumors. Examples of such limited testing would be analogs where the test system of choice is one in the panel, or a compound which is judged to have potential in a solid or slow growing tumor and, therefore, would be tested for biological activity in one or two of the test systems in the panel as well as the P388 leukemia pre-screen. The Colon 38 and Ependymoblastoma tumor test systems are not panel test systems but are included here for information because the numbers were significant and as examples of other test systems utilized by the program.

B. Selection of Candidate Drugs for Clinical Trial

During this report period, 1,319 compounds were added to the Selected Agents list for having demonstrated confirmed in vivo or in vitro activity for the first time. 1,242 (95%) of these new actives were picked up by in vivo tumor systems, with the majority 87% coming through the P388 screen. The remaining 77 compounds were active in the KB cell culture screen (Table 6).

The Data Review Subcommittee (DRS) of the Drug Evaluation Committee (DEC) reviewed the screening results of 1,641 compounds and referred 24 to the DEC. The DEC reviewed a total of 170 compounds. Fifteen new active agents eventually passed the DCT-Linear Array Decision Network point 2A (DN2A) (Tables 7, 8).

C. Detailed Evaluation of New and Old Drugs of Particular Interest

Since the inception of the NCI screening program over twenty years ago approximately 555,000 materials have been screened as possible anticancer agents. Approximately 275,000 of these have been natural products and 280,000 have been synthetic agents. During this report period the new system of pre-selection of synthetic materials has increased to 7.6% the rate of active materials selected by the screen. From among all of the materials tested, 7,520 (1.3%) are currently considered to be of particular interest and the file of these Selected Agent

Compounds (SAC), maintained by the Section, contains comprehensive records of experimental results for active drugs and other materials of special interest. Approximately one-fifth of these are being actively pursued on their own merit and the others are included in comparison studies with related drugs. New data received from this program, other DTP contract or intramural laboratories, non-NCI sponsored drug evaluation programs, and the literature is reviewed and when warranted these materials are presented to appropriate NCI committees for review.

The Screening Section is responsible for the detailed evaluation in rodent tumor systems of drugs which passed DN-2A including route and schedule dpendency, biological quality control of experimental clinical formulations, toxicity in non-tumor bearing rodents, and other appropriate studies to determine the extent to which modifications in drug usage might increase therapeutic efficacy. Results are reviewed and summarized for Decision Points subsequent to DN-2A, for inclusion in clinical brochures and IND applications, and for presentation to clinical working groups. Combination chemotherapy and combined modalities of treatment are evaluated in collaboration with the Office of the Chief, DEB.

1. Route and Schedule Dependency Studies Sixty schedule dependency studies were initiated during the period covering 1976 and the first quarter of 1977: 50 in i.c.-implanted ependymoblastoma, 4 in L1210, 3 in B16, and 3 in other assays.

2. Combination Chemotherapy Studies An offline computer file of animal data for combinations of therapies is maintained and includes a total of 3,402 therapy combinations that have been tested in rodent models; some in several models, in several experiments, and at several laboratories. DEB is currently analyzing these combination therapy studies to attempt to correlate murine data with clinical experience.

3. Biological Quality Control of Experimental Clinical Formulations Comparative testing of bulk drug and various experimental clinical formulations was conducted for eight agents in development to clinical trial. Such testing, which may require the evaluation of a number of experimental forms for each drug, is necessary to insure against loss or reduction of antitumor efficacy during the process of formulation.

4. Screening of Structural Congeners of Active Drugs In vivo testing of congeners was conducted at Arthur D. Little, Inc. (N01-CM5-3765) using animal-tumor models selected on the basis of the qualitative and quantitative activity of the parent compound. While the main objective of this project is to develop congeners with potential clinical superiority, it also provides information to aid in the selection of approaches to new synthesis. Materials are tested within one week of receipt in the laboratory and results are sent

directly to the supplier of the material.

This project is part of the DCT approach to "analog" development and entails (a) identification by DCT of drug classes of special interest, in particular the major classes of clinically active drugs; (b) acquisition, mainly through DCT contracts, of new structural congeners and other rationally-designed drugs; and (c) biological, biochemical, toxicological, and pharmacological comparative testing of congeners to determine potential superiority over the parent drug or uniqueness of action.

D. Methodology (Research and Development)

During this past year, emphasis was placed in the statistical analysis of several tumor models in the testing scheme with the goal of confirming statistical limits, the selection of optimal regimens, the selection of the best host mouse for each system, and the establishment of new protocols. Accordingly, in vivo laboratories conducted several studies which are listed below:

1. A study of the P388 leukemia tumor system was conducted involving six laboratories. The objectives of this study were to determine a) whether three mice per dose level could be substituted for the current six mice per dose level, b) whether the CDF_1 mouse could be substituted for the BDF_1 mouse, and c) to determine the optimal screening regimen. The results of this study indicated that a) the use of six mice per dose level must be continued, b) the CDF_1 mouse could be substituted for the BDF_1 mouse, c) daily treatment days 1 - 9 was the best schedule.

2. There is considerable clinical interest in the efficacy of materials in the mouse ependymoblastoma tumor system. In the interest of economy and the supply of the host mouse, a study of the feasibility of substituting the $B_6C_3F_1$ mouse for the C57Bl mouse as the host was conducted. Preliminary analysis indicates that this substitution can be made.

3. With a murine colon tumor listed as one of the panel tumors, a modest study was conducted using both the C26 and C38 colon tumors in order to establish protocols. Preliminary protocols were established and a larger study is currently in progress in order to statistically validate these protocols.

4. With the introduction of the $B_6C_3F_1$ mouse as a host for the ependymoblastoma system, a study was initiated to determine if this hybrid could be substituted for the BDF_1 hybrid as the B16 melanoma host. This study is currently in progress.

5. The Friend Virus Erythroid Leukemia, an in vitro system in which both cytotoxicity and the erythroid stimulation (indicative of re-differentiation) by materials can be studied, was proposed as

a tumor model for the testing of new synthetic materials. This system has been used in research laboratories and a study was initiated to determine what modifications, if any, were needed in order to include it in a large screening program. However, due to 1) virus shedding by this tumor, 2) the need to use a carcinogen for differential staining, 3) the need for prompt "reading" of the stained cells, 4) the lack of a suitable radioisotope assay that could be used in a mass screening effort and 5) the cost of large scale screening with this model, its use as a screen was not implemented.

This past year considerable progress has been achieved in instituting human tumor xenografts into the tumor panel. A contract was awarded to Battelle Memorial Institute, Columbus, Ohio, (Contract No. NO1-CM6-7099) for the establishment of a xenograft screening laboratory, and a contract was awarded to Stehlin Research Foundation (NO1-CM6-7073) for development of xenograft models and studies of their biological and operational characteristics.

Currently, the established xenograft lines at Battelle Memorial Institute consist of one lung, three breast and three colon tumors. To date, testing in the MX-1 (breast) xenograft has shown that Cytoxan and Melphalan have efficacy whereas BCNU, MeCCNU, 5-FU, AT125, retard growth but do not cause regression. Lymphosarcin and L-alanosine are ineffective. Testing in the CX-2 (colon) tumor revealed that MeCCNU was active and that Cytoxan, Melphalan, 5-FU, Lymphosarcin and Mitomycin C retarded growth but did not cause regression of the tumor. Methotrexate, 5-FudR, AT125, Ara-C, BCNU, and Hexamethylmelamine were completely inactive. In the CX-1 colon tumor, a slower growing tumor than the CX-2, 5-FU, CCNU and MeCCNU were completely inactive in contrast to the results using CX-2. Bleomycin, as tested initially, retarded growth which resumes upon cessation of treatment. Both Acridinyl Aniside (AMSA) and Anguidine were inactive against the MX-1 or CX-2 xenograft tumors. Both Cytoxan and Adriamycin were inactive in the LX-1 (oat cell lung carcinoma). In addition to the methodology previously listed, individual laboratories conducted methodology research under the direction of the project officer.

a) Battelle Memorial Institute, Columbus, Ohio (NO1-CM5-7005)

Caloric restriction studies were conducted in both the murine colon tumors No. 26 and No. 38. It was noted that caloric restriction resulted in shortened survival time of mice with colon 26. However, mice with colon 38 and fed 4 grams daily had a 29% reduction in tumor size compared to that of the mice fed *ad libitum*. A reduction below 4 grams of food resulted in significant animal weight loss.

b) IITRI (NO1-CM5-7004)

Studies were continued with two lines of a murine ascitic tumors acquired from the USSR. To date reproducible survival times have not been achieved. A solid murine squamous cell carcinoma of the cervix, also from the USSR, was studied. Due to its approximate survival time (>60 days) when implanted subcutaneously, plus the ulceration after 5 - 6 weeks, this tumor, when inoculated by this route, appears unsuitable for large-scale screening. Studies are in progress to determine if better results can be obtained when the tumor is implanted intraperitoneally.

Caloric restriction studies in both colon 38 and the CD, mammary carcinoma were conducted. Only when there was a weight loss of $\geq$ 5 grams was there a significant reduction in size of colon 38. Caloric restriction for 10 days ($\leq$ 3 gm./day) resulted in a significant decrease in the size of the CD, mammary tumor. These studies are continuing.

c) Arthur D. Little, Inc. (NO1-CM5-3765)

With the increased emphasis in drug combinations and combined modalities by clinicians, research was conducted in these areas.

1) Drug Combination: Sixty drug combinations have been conducted in either ascitic or solid tumors. Instances where the combination was superior to either of the individual drugs are listed below. The combination was considered superior if either or both of the following criteria was observed, a) the combined T/C % value is greater than 25% above % T/C value of either drug alone, b)

$$\text{Combined \% T/C} > \frac{\text{\% T/C}_{(A)} \times \text{\% T/C}_{(B)}}{100}$$

Drugs			Test System
Methotrexate	plus	Chlorambucil	P388
"	"	Mitomycin C	L1210
"	"	CCNU	L1210
6-Mercaptopurine	"	Vinblastine	L1210
Merchlorethamine	"	Hydroxyurea	L1210
"	"	Dibromodulcitol	L1210
Actinomycin D	"	Adriamycin	L1210
Chlorambucil	"	Mitomycin C	P388
Melphalan	"	5-Flurouracil	L1210
Cytoxan	"	BCNU	L1210
Mitomycin C	"	Cytoxan	B16
Hydroxyurea	"	Cytoxan	Lewis Lung
"	"	Vinblastine	P388
Vinblastine	"	Vincristine	P388
"	"	Procarbazine	P388

1) Tumor Growth Kinetics: Studies of the growth characteristics of the Lewis Lung and Ridgway osteogenic sarcoma have been previously reported. This past year the different responses to known drugs by these two tumors was analyzed. The Ridgway osteogenic tumor has striking drug-induced regressions that do not occur in the Lewis Lung tumor. These new studies appear to indicate that after drug treatment there was a rapid removal of cellular debris, etc. from the tumor site followed by a long delay in tumor regrowth, a reflection of the long doubling time of the remaining small tumor mass. In addition, there was a lengthening of tumor cell cycle in the Lewis Lung tumor as the tumor plateaus, whereas in the Ridgway, the cell cycle was longer during the period of early growth. This resulted in less time for the host to recover from therapy before the tumor attained pre-treatment size in the Lewis Lung tumor as compared to the Ridgway tumor, possibly resulting in different efficacies.

The growth kinetics of Lewis Lung secondary foci following surgical removal of the primary tumor was studied. Surgery (including sham) increased the thymidine index of these foci whereas the presence of the primary tumor, suppressed the thymidine index of the secondary foci. Thus, chemotherapy should be initiated as soon as possible following surgery in this system.

III. Related Projects

The need for uniformity among contract laboratories in a large screening program requires continued surveillance. It is the responsibility of the Screening Section to assure uniform biological characteristics of tumor lines as well as host animals. To achieve this, frozen tumor banks are maintained by several contract laboratories from which new lines are dispensed periodically to screening laboratories.

The need for host animals in good health and in large numbers necessitates close liaison with the Mammalian Genetics and Animal Production Section, DTP, whose contract laboratories perform studies related to the diagnosis and control of animal disease and monitor the quality of the animals utilized in the program.

The Screening Section, as the major user of the DTP data processing system, continues to work closely with the Automated Information Section, DEB, by advising and assisting in the planning of modifications and the monitoring of data necessary to an ongoing data processing system.

IV. Proposed Course of Projects

The Screening Section will continue to supervise the testing and evaluation of data for all materials screened in the DTP program. This

Drugs			Test System
Vincristine	plus	Adriamycin	P388
"	"	BCNU	L1210
Procarbazine	"	BCNU	L1210
CCNU	"	Dibromodulcitol	L1210
Bleomycin	"	Cytoxan	Lewis Lung
"	"	Vinblastine	B16
"	"	Cis - Platinum II	B16

2) Surgical Adjuvant Chemotherapy: It has been previously reported by several investigators that surgical removal of a leg implanted with the Lewis Lung tumor prior to Day 6 significantly increased the survival time of mice. If amputation was performed on Day 10 or later, surgery was not effective in significantly increasing life span. Nine clinically active drugs were assayed for efficacy in this modified system. BCNU, MeCCNU, and Cytoxan were markedly effective, Bleomycin was moderately effective, Vincristine and Hexamethylmelamine were marginally effective. Methotrexate, Adriamycin, and Nitrogen mustard were not effective. An interesting observation was that BCNU or MeCCNU given a few days prior to surgery was effective although both agents are reported to be immunosuppressive drugs.

γ radiation to the thoracic region, post surgical removal of the tumored leg, was effective in increasing life span.

d) Mason Research Institute (N01-CM5-7003)

During 1976 and the first quarter of 1977 requests were received for testing in non-protocol tumor systems. The number of materials tested in mouse systems are as follows: 73 in the M5076 ovarian carcinoma, 60 in the FCB bladder carcinoma, 12 in the C1498 myleoid leukemia, 25 in the FV01Y Friend virus leukemia, 9 in the M4898 papillary adenocarcinoma of the lung, and 1 in the M5480 testicular carcinoma. Requests were also received for testing in non-protocol rat tumor systems and the number of materials for each system are as follows: 2 in the 11095 prostatic carcinoma, 1 in the R3327 prostatic carcinoma, 3 in the 13762 mammary adenocarcinoma, 1 in the 13762E mammary adenocarcinoma, and 2 in the R3230AC mammary adenocarcinoma.

e) Southern Research Institute (N01-CM4-3756)

Methodology research conducted at the Southern Research Institute is multifaceted. The major areas investigated, and the findings are as follows:

includes the continuous evaluation of the primary screen and its logistics, criteria, and tumor models. Research and methodology will be carried out under contract for related projects of interest to DCT, NCI. Research projects are aimed primarily at devising new or improved antitumor assays, evaluation of new test systems for their potential as screening tools, and the development of standardized methodology to be used by the participating contract laboratories.

TABLE 1

NUMBER OF IN VIVO AND IN VITRO SCREENING TESTS CONDUCTED IN CALENDAR YEARS 1976/77*

	Selected Agents Compound (SAC)		Other Synthetics		Natural Products			
	Regular	Analog	Regular	Analog	Plants	Animals	Fermentations	Total
1976								
1st Quarter	8,515	1,775	23,696	1,268	15,337	3,797	2,142	21,276 (Crude)
					10,076	2,063	3,171	15,310 (Fraction)
					25,413	5,860	5,313	36,586 (Both)
2nd Quarter	7,806	2,757	19,858	1,228	14,670	1,826	2,303	18,799 (Crude)
					6,291	2,850	3,403	12,544 (Fraction)
					20,961	4,676	5,706	31,343 (Both)
3rd Quarter	9,463	2,202	25,782	963	15,982	2,156	2,857	20,995 (Crude)
					8,651	1,631	2,939	13,221 (Fraction)
					24,633	3,787	5,796	34,216 (Both)
4th Quarter	8,797	2,267	23,718	1,048	10,421	3,983	2,702	17,106 (Crude)
					6,777	1,917	3,607	12,301 (Fraction)
					17,198	5,900	6,309	29,407 (Both)
Total	34,581	9,001	93,054	4,507	88,205	20,223	23,124	131,552 (Both)
1977								
1st Quarter	6,104	1,663	26,510	1,188	15,265	1,558	3,047	19,870 (Crude)
					4,318	1,603	3,576	9,497 (Fraction)
					19,583	3,161	6,623	29,367 (Both)

	1st Quarter 1976	2nd Quarter 1976	3rd Quarter 1976	4th Quarter 1976	1976	1st Quarter 1977
Total All Tests	71,840	62,992	72,626	65,237	272,695	64,832

*Includes tests begun no earlier than January 1, 1976, and reported to the Automated Data Processing (ADP) Contractor by May 2, 1977.

TABLE 2

NUMBER OF IN VIVO SCREENING TESTS CONDUCTED IN CALENDAR YEARS 1976/77*

	Selected Agents Compound (SAC)		Other Synthetics					
	Regular	Analog	Regular	Analog	Plants	Animals	Fermentations	Total
1976								
1st Quarter	8,400	1,775	23,288	1,268	13,161	3,048	1,645	17,854 (Crude)
					7,719	636	3,127	11,482 (Fraction)
					20,880	3,684	4,772	29,336 (Both)
2nd Quarter	7,646	2,757	19,663	1,228	12,458	1,560	1,512	15,530 (Crude)
					4,493	1,137	3,336	8,966 (Fraction)
					16,951	2,697	4,848	24,496 (Both)
3rd Quarter	9,349	2,202	25,636	963	13,241	2,023	2,113	17,377 (Crude)
					6,252	790	2,844	9,886 (Fraction)
					19,493	2,813	4,957	27,263 (Both)
4th Quarter	8,654	2,267	23,466	1,048	7,577	2,980	2,080	12,637 (Crude)
					4,468	741	3,306	8,515 (Fraction)
					12,045	3,721	5,386	21,152 (Both)
Total	34,049	9,001	92,053	4,507	69,369	12,915	19,963	102,247 (Both)
1977								
1st Quarter	5,994	1,663	26,342	1,118	11,980	1,469	2,234	15,683 (Crude)
					2,535	460	2,902	5,897 (Fraction)
					14,515	1,929	5,136	21,580 (Both)

	1st Quarter 1976	2nd Quarter 1976	3rd Quarter 1976	4th Quarter 1976	1976	1st Quarter 1977
Total All *In Vivo* Tests	64,067	55,790	65,413	56,587	241,857	56,697

*Includes tests begun no earlier than January 1, 1976, and reported to the ADP Contractor by May 2, 1977.

TABLE 3

NUMBER OF IN VITRO SCREENING TESTS CONDUCTED IN CALENDAR YEAR 1976/77*

	Selected Agents Compound (SAC)		Other Synthetics		Natural Products			Total
	Regular	Analog	Regular	Analog	Plants	Animals	Fermentations	
1976								
1st Quarter	115		408		2,176	749	497	3,422 (Crude)
					2,357	1,427	44	3,828 (Fraction)
					4,533	2,176	541	7,250 (Both)
2nd Quarter	160		195		2,212	266	791	3,269 (Crude)
					1,798	1,713	67	3,578 (Fraction)
					4,010	1,979	858	6,847 (Both)
3rd Quarter	114		146		2,741	133	744	3,618 (Crude)
					2,399	841	95	3,335 (Fraction)
					5,140	974	839	6,953 (Both)
4th Quarter	143		252		2,844	1,003	622	4,469 (Crude)
					2,309	1,176	301	3,786 (Fraction)
					5,153	2,179	923	8,255 (Both)
Total	532		1,001		18,836	7,308	3,161	29,305 (Both)
1977								
1st Quarter	110		168		3,285	89	813	4,187 (Crude)
					1,783	1,143	674	3,600 (Fraction)
					5,068	1,232	1,487	7,787 (Both)

	1st Quarter 1976	2nd Quarter 1976	3rd Quarter 1976	4th Quarter 1976	1976	1st Quarter 1977
Total All In Vitro Tests	7,773	7,202	7,213	8,650	30,838	8,065

*Includes tests begun no earlier than January 1, 1976, and reported to the ADP Contractor by May 2, 1977.

TABLE 4

SUMMARY OF MATERIALS FIRST TESTED IN ANY SYSTEM IN 1976/77*

	Selected Agents Compound(SAC)			Other Synthetics			Natural Products				
Quarter	Synthetic	Analog	Total	Synthetic	Analog	Total	Plants	Animals	Fermen-tations	Total	Total
1976											
1	180	45	225	5,017	141	5,158	1,835	596	449	2,880	8,263
2	225	68	293	4,158	185	4,343	2,064	213	393	2,670	7,306
3	141	38	179	5,893	125	6,018	2,236	326	405	2,967	9,164
4	102	32	134	5,434	157	5,591	1,376	736	489	2,601	8,326
Total	648	183	831	20,502	608	21,110	7,511	1,871	1,736	11,118	33,059
1977											
1	8	0	8	5,304	149	5,453	2,272	165	490	2,927	8,388

Analysis of lag time between the date test initiated at the laboratory (date on) and the date the test results are processed, reveals that the following percentages of the data have been processed in the second, third, and fourth months after the date-on month:

Month Beyond Date-on	SAC		Synthetics		Natural Products	
	Average	Range	Average	Range	Average	Range
Second	23%	8%-53%	36%	22%-65%	64%	25%-84%
Third	66%	53%-79%	75%	43%-98%	96%	91%-99%
Fourth	92%	86%-96%	92%	68%-99%	99%	98%-100%

*Includes compounds for which test results were processed by May 2, 1977.

TABLE 5

NUMBER OF TESTS CONDUCTED AGAINST PANEL TUMOR SYSTEMS IN CALENDAR YEARS 1976/77*

Panel Tumor Systems	1st Quarter 1976	2nd Quarter 1976	3rd Quarter 1976	4th Quarter 1976	1976	1st Quarter 1977
L1210 Lymphoid Leukemia (LE)	4,939	4,331	3,008	2,407	14,685	1,777
B16 Melanocarcinoma (B1)	1,936	1,129	1,739	1,935	6,739	1,507
Lewis Lung Carcinoma (LL)	777	559	297	277	1,910	455
$CD8F_1$ Mammary Tumor (CD)	33	120	96	209	458	336
Colon 26 (C6)	6	241	938	1,263	2,448	726
Colon 38 (C8)	16	36	384	673	1,109	1,083
Ependymoblastoma (EM)	592	274	1,971	1,203	4,040	902
Total All Panel Tumor Systems	8,299	6,690	8,433	7,967	31,389	6,786

*Includes tests begun no earlier than January 1, 1976, and reported to ADP Contractor by May 2, 1977.

TABLE 6

New Confirmed Active Compounds added to the Selected Agents Listing

For 1976 and 1st Quarter of 1977

Total Number of Materials	Number of Materials by Tumor System
1,242 *in vivo*	1,070 P388 Leukemia
	147 L1210 Leukemia
	3 W256 - IM
	10 W256 - IP
	7 B16 Melanoma
	1 Gardner Lymphosarcoma
	1 L-5178Y Lymphatic Leukemia
	2 Lewis Lung
77 *in vitro*	77 KB cell culture

TABLE 7

Status of Active Compounds

For 1976 and 1st Quarter of 1977

New Confirmed Actives	1,319
Compounds Reviewed	
DRS	1,641
DEC	170
Total	1,811
Passed DN2	15
Route and Schedule Dependency Studies	
Scheduled	62
Reviewed by Screening Committee	39

TABLE 8

COMPOUNDS ASSIGNED TO DN-2 FOR THE FIRST TIME IN 1976
AND DURING THE FIRST QUARTER OF 1977

NSC NO.	NAME	DATE OF ASSIGNMENT	BASIS FOR ASSIGNMENT (ACTIVITY)
118742	Melamine, *N*2,*N*2,*N*4,*N*4,*N*6-pentamethyl-, monohydrochloride	Jan. 17, 1977	Human lung tumor xenograft
141401	Poly(*p*-dioxane-2,5-diyloxyethyleneiminohexamethyleneiminoethyleneoxy)	Dec. 13, 1976	B16 melanoma
153353	Propionic acid, 2-amino-3-(hydroxynitrosamino)-, monosodium salt Alanosine, monosodium salt	May 14, 1976 (per O.C.)	L1210 leukemia
164011	Daunorubicin, benzoylhydrazone, monohydrochloride Rubidazone	June 14, 1976	P388 leukemia (reported to have less cardiac toxicity than Adriamycin--(NSC-123127)
177023	Imidazo [2,1-*b*] thiazole, 2,3,5,6-tetrohydro-6-phenyl-, monohydrochloride Levamisole	Aug. 16, 1976	Already in clinical use. NCI is to cross file IND
182986	1,4-Cyclohexadiene-1,4-dicarbamic acid, 2,5-bis(1-aziridinyl)-3,6-dioxo-diethyl ester AZQ	June 14, 1976	IC-L1210 leukemia IC-Ependymoblastoma

TABLE 8

NSC NO.	NAME	DATE OF ASSIGNMENT	BASIS FOR ASSIGNMENT (ACTIVITY)
191959	High MW Glycoprotein	Mar. 22, 1976	B16 melanoma P388 leukemia
218321	Imidazo[4,5-d][1,3]diazepin-8-ol, 3-(2-deoxy-β-D-erythro-Pentofuranosyl)-3,4,7,8-tetrahydro- 2'-Deoxycoformycin (ADI)	Jan. 26, 1976	Adenosine deaminase inhibitor; potentiator of in vivo activity of adenine arabinoside.
219877	Rachelmycin	Aug. 16, 1976	B16 melanoma P388 leukemia
240899	5-Benzofurancarboximidamide, 2,2'-(1,2-ethenediyl)bis-, dihydrochloride	Mar. 22, 1976	L1210 leukemia
259968	Compound from Bouvardia Ternifolia	Mar. 1, 1977	B16 melanoma
263164	9H-Purine-9-ethanol, 6-amino-β-hexyl-α-methyl-, monohydrochloride STE (R*, S*)-	Dec. 13, 1976	Adenosine deaminase inhibitor; potentiator of in vivo activity of adenine arabinoside.
264880	1,3,5-Triazin-2(1H)-one 4-amino-3,6-dihydro-1-B-D-ribofuranoyl-monohydrochloride Dihydro-5-azacytidine, 5-AC[H]	June 14, 1976	Solution Stability Better than NSC-102816. Active (T/C of 216 and 228%) in 3LE21 IP QD 1-9 at 100 mg/kg/injection
282175	4-Hydroxyandrost-4-ene-3,17-dione	Jan. 17, 1977	Rat Mammary Cancer at Worcester Foundation for Exp. Biology
283162	Methanol, [1,3,5-triazine-2,4,6-triyltris(methylimino)]tris-	Jan. 17, 1977	Human lung tumor xenograft

ANNUAL REPORT
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DIVISION OF CANCER TREATMENT
NATIONAL CANCER INSTITUTE

Since cancer is not one disease but a number of diseases affecting the different organs and systems of the body, many different approaches to cancer treatment may be adopted. The major approaches include surgery, radiation, chemotherapy, immunotherapy, or a combination of the four. Research in drug development includes selection and procurement of agents for testing, bioassay of the agent in tumor systems, preclinical pharmacological and toxicological study, and clinical evaluations. The foundation of chemotherapy is the drug itself - either single or in drug combination. Consequently, the search for more effective, less toxic and broader acting agents will continue.

Past experience has clearly demonstrated that effective antitumor agents are not restricted to a single, or even a few chemical classes but that the chemical-structural parameters are broad. One must conclude then that all effective agents are not operating by a single mechanism. Therefore, efforts are directed towards the chemical modifications of existing drugs to increase their activity and/or decrease their toxicity, and to search for new agents via chemical synthesis, or from natural sources.

Materials from these sources will be evaluated in the primary screen and those agents demonstrating sufficient activity will be subjected to toxicological studies and possible clinical evaluation.

Research efforts are, in large measure, based on leads provided by the primary screens, biochemical and toxicological studies, pharmacokinetics observations, literature reports, and the surveillance of the worldwide scientific community for information on new drugs. As the vast storehouse of information accumulates and greater achievement is being experienced in the control of the more rapidly proliferating phase of cell growth or young tumors, the search for new structures aimed against the slower growing tumors in specific organs, i.e., lung, breast, colon, brain, etc. will be the prime objectives.

During this report period a major reorganization of the Drug Synthesis and Chemistry Branch (formerly the Drug Development Branch) was finalized. Two new branches were created, namely, the Natural Products Branch and the Pharmaceutical Resources Branch.

Branch Staff

The Drug Synthesis and Chemistry Branch is composed of a staff of persons consisting of five senior professional scientists (GS-13 and above), six other professional staff members (GS-9 to GS-12) and the remainder (7) technical and clerical staff.

Those responsible for the activity of the two Sections are as follows:

Drug Synthesis & Chemistry Branch
Chief -- Dr. Harry B. Wood, Jr.

Dr. Synthesis Section
Head, Dr. Venkatachala L. Narayanan

Chemical & Drug Information Section
Mr. Sidney Richman

The Acquisition of Chemicals Unit (Head, Mr. Robert B. Ing) operates as an activity from the Office of the Chief of the Drug Synthesis & Chemistry Branch.

Objectives

As part of the broad Division of Cancer Treatment Program, the Drug Synthesis and Chemistry Branch has three major objectives:

I. To develop new drugs via a chemical contract synthesis program.

II. To acquire new agents for screening via acquisition from the scientific-community-at-large.

III. To develop, maintain and utilize a chemical information system.

Implementation of Program

These objectives are carried out through the activities of the Branch's Sections which are:

I. The Drug Synthesis Section: The objectives are divided into the design and synthesis of new drugs. The thrust is directed towards analog improvement, i.e., broader spectrum of activity, and decreased toxicity, and the development of new exploratory leads and the synthesis of unique structures as potential anticancer agents.

Even though major advances have been made in recent years, the treatment of cancer is still limited by the availability of useful agents. The usefulness of the majority of the present agents are limited also by severe toxic side effects and/or are associated with the development of drug resistant tumors. Therefore, a greater variety of agents with diverse structures and different mechanism of action, broader spectrum of activity and a greater safety would be extremely valuable in cancer treatment. The problem of finding these improved drugs is the main objective of the contract synthesis program and its efforts are channeled into many areas of investigation. As the rapidly growing tumors become more and more under control, attention is being focused upon developing new drugs for the slower growing tumors, especially for lung, breast, colon, etc.

II. Chemical and Drug Information Section: The objective of the Section is to develop and operate a data processing system and to devise and use various computerized techniques to meet the scientific, operational control and management planning needs of the Branch. The Section is concerned with: (1) operating, maintaining, and enhancing the automated Drug Synthesis and Chemistry's Chemical Information System which supports the accessioning, storage, and distribution of chemicals and drugs acquired for antitumor testing, including the coordination of staff and contract activities related to CIS; (2) managing the storage and retrieval of chemical information in both automated and manual DTP files; (3) conducting structure-activity studies to optimize acquisitions by identifying chemical classes to be deemphasized (because they are largely negative or adequately studied) and by selecting new accessions from among potential acquisitions, aided by a developing model which assigns activity scores in the P388 lymphocytic leukemia prescreen to such compounds; and (4) identifying for acquisition compounds of interest which are reported in the literature or are in various chemical files maintained by governmental agencies, industrial organizations, and information venders.

III. Acquisition of Chemicals (Office of the Chief): The objectives of this effort are to insure the continuous flow of approximately 15,000 new preselected synthetic compounds annually for the primary screen and to acquire approximately 500 to 1000 samples for the tumor panel. Extensive efforts are maintained with industry, government laboratories, and with the scientific public in academic and research institutions. Effort is directed toward (1) developing and maintaining sources for preselected compounds with unique structural features and biological activity; (2) the acquisition of compounds for the primary screen and tumor panel evaluations; (3) developing compound selection criteria; (4) conducting structure-activity correlation studies; (5) monitoring the discreet agreements with industrial suppliers; and (6) providing for the proper storage and distribution of all accessioned materials.

Operation of Program

The methods for selecting new agents for input into the primary screen are gradually changing. More emphasis is being directed toward selectivity of agents and less emphasis towards random procurement. This should result in more active analogs of existing active materials, fewer biological tests required, and logically, the number of active agents should be higher. Acquiring such preselected agents may be more costly. Changes in the screening protocol should be incorporated to include differentiation of analog activity, including toxicological evaluations where appropriate.

During 1976 the majority of exploratory compounds were acquired from industrial research sources but selected on criteria developed by the NCI staff. This selection process resulted in increasing the number of actives to 7.2% which previous submissions provided only 1.5% actives. However, as preselection increases and as the structure-activity mosaic gradually takes shape, the choice of chemical compound for introduction to the screen will emerge more from directed synthesis and will gradually replace random procurement. The gradual mutation from random input to directed selection of agents for screening will be generated from review and evaluation of chemical and biological data from within the program, in the literature and other related programs. Consideration is directed toward antitumor structure-activity relationships with emphasis on new and promising structural classes and deemphasis or elimination of adequately studied classes or non-productive structural classes. Unfortunately, the state-of-the-art has not reached a degree of sophistication which allows a completely rational approach to drug design.

One major thrust of analog synthesis will be in the area of clinically effective drugs. Analogs will be designed for therapy improvement and decreasing the undesirable features of the parent drug. Also analog synthesis will be aimed at improving those agents which have shown therapeutic effectiveness in the screen but have been abandoned later because of toxicological or other problems. Synthetic analogs of known active drugs may possess superior properties because of better transport and greater resistance to degradation or greater binding to appropriate enzyme sites. Synthetic analogs may produce longer or shorter duration of activity as the needs dictate, alter the solubility characteristics and improve the stability. Structural modifications could also introduce new chromophore groups to aid in determining the drug level in the host. Analogs would also be used in mechanistic studies, and biochemical investigations which include areas of whole body drug distribution, drug metabolites, enzymatic activity, and the route and rate of excretion.

New chemical structures or analogs, developed through biochemical cell cycle kinetic studies, pharmacological and pharmaceutical rationales, and biological activities demonstrated in non-cancer programs, should provide additional input to the directed preselection and synthesis approach. Plans are underway to group together those agents which have shown no cross-resistance and determine if there are any structural similarities for future analog design. The same study will be made on those agents which have shown potentiations in classical biological and/or biochemical screens.

The major synthetic activity will be towards broader acting drugs. The search for agents which have a high degree of organ localization will be undertaken and compounds containing this property should be considered as potential leads for chemical modification. A program to synthesize drugs localizing in the lung, breast, colon, etc. will be considered and to modifying their chemical structure with the objective of introducing antitumor properties. Organ specific screens are needed to evaluate such new types of agents. The increasing awareness that many antitumor drugs may possess latent carcinogenic properties will be considered in the hopes of eliminating such characteristics. Synthetic development may provide improved methods of synthesis which would achieve economic advantages in the production of large amounts of drugs for the clinic, i.e., methotrexate during this past period.

Chemical modification and structural analogs of natural products will also provide unique compounds of interest. The continued follow-up of empirical observations and changes in the structure of the already effective agents will also add to our array of new material for investigations. The random fermentation products, algae, marine and insect products will continue to be explored.

The Branch is keenly interested in new approaches to drug design. One such approach being exploited is the synthesis of analogs to develop and exploit structure-activity relationships. When a significant new activity has been established, there is need for a major effort to prepare and study related derivatives for the purpose of defining the relation of structure to biological activity. It is desirable to study a series of closely related active compounds to determine toxicity, transport, and other chemo-physical properties. Recognizing that such techniques are still in the early stages of development, effort will continue to improve and develop new methodologies. Several problems do still exist in this area of investigation; such as obtaining the physical-chemical measurements which are quite exact whereas the biological testing data has a considerable amount of variation. It must be recognized that this approach may not necessarily give immediate answers since it may be necessary to develop new parameters. However, it is hoped that such studies will focus attention on synthetic areas of greatest potential and provide the chemist with more directed synthetic approaches. This is not to be interpreted that new structural classes will be by-passed.

Worldwide Surveillance

An extensive liaison is maintained between the Branch and the pharmaceutical and chemical industries, grantees and academic institutions, research organizations and other governmental laboratories, since a substantial number of compounds are contributed to the Program on a voluntary basis from these sources. Good public relations must be and have been maintained by reporting of data and by making special handling of unusual agents a matter of normal service. With the increasing complexity of data processing and growth of the Program, constant awareness of the problems of 'Commercial Discreet' data must ever be present. A total of 3807 individual suppliers, of which 1528 are industrial suppliers, have contributed samples to the Program. During the reporting period there have been 279 new non-industrial suppliers and 131 new industrial suppliers submitting materials for primary screening. Even though the majority of these contributors are domestic suppliers many materials have been supplied from foreign countries. The increasing number of contributors is an index of the continued enthusiasm for the Program by the scientific community.

The Branch's continuous international surveillance for acquiring new agents, synthetic and natural products, has provided over 833 foreign suppliers from industry, academic institutions and governmental and research organizations. Close contact is maintained with these and other potential new suppliers to provide interchange of chemical data and biological information on potential chemicals and drugs of interest to the NCI and the scientific community-at-large.

The Branch has 26 contracts; Twenty-two synthesis contracts, see Table VI under Drug Synthesis Section; two chemical informations contract, see

Table XI under Chemical Drug Information Section; and two miscellaneous contracts, see Table I below under Miscellaneous Branch Contracts.

Table I

MISCELLANEOUS BRANCH CONTRACTS

CONTRACTOR	INVESTIGATOR	CONTRACT NUMBER
Microbiological Associates, Inc.	Mozejko	NO1-CM-43793
Starks C. P., Inc.	Paull	NO1-CM-53745

The Starks C. P., Inc. activities include: (1) selective acquisition of novel chemicals and drugs for the primary screen, (2) serves in a liaison capacity to develop new sources of compounds, (3) monitors chemical literature for unique structures of interest, (4) prepares the necessary documentation for the computer registration of samples, (5) monitors the flow of computer generated out-puts to suppliers and to management, (6) maintains accurate records of all transactions dealing with liaison activities with suppliers, the operation contractor for the Chemical Information System and with the contractor for storage and distribution of chemicals. The second miscellaneous contract, Microbiological Associates, Inc., provides the Division of Cancer Treatment with services for the storage of bulk chemicals and for the distribution of these materials to program research laboratories throughout the world. The contractor also maintains an inventory and shipping record for these bulk materials. Custody and records of the 'Drug Abuse' materials are also maintained.

New Areas of Investigation

I. Development of Radiosensitizers

The treatment of solid tumors via radiation does not always eradicate the total local tumor cell population. It is recognized that the inner core of solid tumor mass has a decreased blood supply and consequently a decreased oxygen supply. Cells deficient in oxygen content (hypoxic cells) are able to resist the killing affect of radiation. Some classes of chemicals, those with electron affinity, appear to mimic the effect of oxygen in the cell. The resistance may be decreased by the use of drugs that can sensitize the cells to radiation and assist in eliminating the tumor burden. Therefore a contract effort is being developed with the objective of synthesizing compounds which will (a) diffuse readily into tumor cells, especially cells wtih a poor blood supply, (b) metabolize slowly to non-toxic metabolites, and (c) sensitize cells to radiation but not inhibit the repair mechanism of normal cells. Preliminary studies suggest several types of compounds as potential candidates for this work; those include nitroimidazoles, nitrothiazoles, nitropyrimidines, thiosugars, etc. Such compounds will be evaluated for electron affinity, partition coefficient, *in vitro* and *in vivo* effects upon radiation of tumors with hypoxic cells.

II. Development of Methods for Preselection of Compounds for the Preliminary Screen

The study and analysis of the chemical file combined with broad structure activity relationship is providing valuable guides for preselecting compounds for the screen. During this past report period these guides have increased the number of actives from 1.5% to 7.2%. This is a major improvement and as the methodology is refined there is every reason to expect this rate to increase. Fortunately this increase has also provided some new classes of chemicals. Some of the new guides include: (a) deemphasize compounds with less than 12 carbons with only one functional type, (b) select compounds with quaternary nigrogen, (c) select compounds wih phosphonium ions, (d) select compounds possessing the "ABONS" criteria, i.e., aromatic benzylic group with an electron rich substituents and with an oxygen, nitrogen, or sulfur in alpha position of the side chain, (e) deemphasize hydrocarbons, (f) deemphasize poly halogen aliphatic and aromatic compounds.

Accomplishments

The Drug Synthesis and Chemistry Branch provided a steady flow of materials into the Program. These materials (see Table II) consisted of chemicals, plant products, marine and insect products and fermentation products. Some of these materials have demonstrated confirmed activity in one or more of the primary screens (Step III) and have been assigned as candidates for preclinical toxicological evaluation (Decision Point IIA) (see Table IV). Six Investigational New Drug Applications (IND) have been submitted to the Food and Drug Administration (see Table II and V). Of the 20233 new crystalline materials added to the Program, 1197 compounds (5.91%) were acquired under contract, 208 compounds (1.03%) were purchased, 15,299 compounds (75.61%) were voluntarily submitted from industrial sources, 3529 compounds (17.45%) were from non-industrial suppliers. Of the 20,233 compounds received; 1,470 were confirmed actives which represents 7.26% actives (see Table III).

The Branch manages a total of 26 contracts with industrial organizations, academic and research institutions, both domestic and foreign, totaling approximately 4.6 million dollars and covering the major areas as shown under the primary responsibilities of the Branch. Of the 26 contracts, 22 are domestic and 4 are foreign contracts.

In summary the following were achieved during this report period year by the Drug Synthesis and Chemistry Branch.

1. A total of 32961 new substances (Table II) was obtained for screening or other evaluation;
    a. 19837 synthetic
    b. 2518 antibiotic cultures
    c. 8673 plant materials
    d. 1537 animal materials
    e. 396 other crystalline materials

2. A total of 1197 new compounds were prepared under contract; 844 from syntheses, and 353 from the prep labs and natural product contracts.

3. Fifteen new substances were assigned to Decision Network IIA (Table II and (IV) (candidates for preclinical toxicology);

   a. 4 fermentation products

   b. 1 plant product

   c. 10 synthetic compounds

4. Six drugs were assigned IND numbers (Table II and V);

5. One thousand four hundred and seventy compounds were assigned to Step III (Table II);

6. Using preselection technique for compound selection for the screen provided a major increase in the number of actives from 1.5% to a new high of 7.2%;

7. Twenty-six contracts were supervised by the Branch;

   a. 22 synthesis laboratories

   b. 4 miscellaneous

8. There was 6 grant proposals and 4 patent applications reviewed and commented upon;

9. Ten patents issued to our contractors, and one by staff;

10. Samples were received from 131 new industrial concerns and 279 new non-industrial sources;

11. One hundred and seventy manuscripts by the DCT contractors were cleared for publication;

12. Sixty-five were published by the Branch contractors and four by staff;

13. One work session and three seminars were sponsored by the Branch.

TABLE II

MATERIAL ACCESSIONED AND ACTIVITY REPORT (1)

	CRUDE EXTRACTS				CRYSTALLINE				DECISION		IND	
	NO. SUBMITTED		NO. ACTIVE		MATERIAL SUBMITTED		STEP III(2)		POINT II(3)		FILED	
	1975	1976-7*	1975	1976-7*	1975	1976-7*	1975	1976-7*	1975	1976-7*	1975	1976-7*
Fermentation Products	1328	2518	141	92	159	217	29	19	3	4	1	-
Animal Products	2086	1537	102	90	38	39	8	-	1	-	-	-
Plant Products	6393	8673	346	567	155	140	30	39	1	1	1	-
Synthetics	-	-	-	-	20943	19837	692	1412	13	10	2	6
Total	9807	12728	589	749	21295	20233	759	1470	18	15	4	6

(1) Based on Calendar Year
* Through March of 1977 (January 1976 through March 1977)

(2) Showing Confirmed Activity

(3) Selected Candidates for Preclinical Toxicological Evaluation and Clinical Trial

TABLE III

SOURCES OF COMPOUNDS

Source	Numbers Accessioned	Number of Actives(*)	Percent Active Total Filed
Non-Industrial	2,916	413(17.66%)**	2.03
Industrial	15,299	649(4.24%)	3.26
Government	613	123(20.06%)	0.59
Contract	1,197	267(22.30%)**	1.30
Purchase	208	18(8.65%)	0.08
Total	20,233	1,470	7.26

* DN-1 Compounds

** High percentage of analogs in group

TABLE IV

COMPOUNDS PASSING DECISION NETWORK IIA

NSC NO.	COMPOUND NAME	SOURCE
118742	Melamine, *N*2,*N*2,*N*4,*N*4,*N*6-pentamethyl-, monohydrochloride	Synthetic
141401	Poly(*p*-dioxane-2,5-diyloxyethyleneiminohexamethyleneiminoethyleneoxy)	Synthetic
153353	Alanosine, monosidium salt	Fermentation
164011	Rubidazone	Synthetic
177023	Levamisole	Synthetic
182986	1,4-Cyclohexadiene-1,4-dicarbamic acid, 2,5-bis (1-aziridinyl)-3, 6-dioxo-diethyl ester	Synthetic
191959	Michicarcin	Fermentation
218321	Imidzao[4,5-*d*][1,3]diazepin-8-ol, 3-(2-deoxy-β-*D*-*erythro*-Pentofuranosyl)-3,4,7,8-tetrahydro-	Fermentation
219877	Rachelmycin	Fermentation
240899	5-Bnzofurancarboximidamide, 2,2'-(1,2-ethenediyl) bis-, dihydrochloride	Synthetic
259968	Compound from *Bouvardia Ternifolia*	Plant
263164	9*H*-Purine-9-ethanol, 6-amino-β-hexyl-α-methyl-, monohydrochloride STE (R*,*S**)-	Synthetic
264880	1,3,5-Triazin-2(1*H*)-one 4-amino-3,6-dihydro-1-β-*D*-ribofuranoyl-monohydrochloride	Synthetic
282175	4-Hydroxyandrost-4-ene-3,17-dione	Synthetic
283162	Methanol, [1,3,5-triazine-2,4,6-triyltris(methylimino)]tris	Synthetic

TABLE V

IND'S FILED

NSC NO.	COMPOUND NAME	SOURCE
19987	Methylprednisolone	Synthetic
73754	Fluorodopan	Synthetic
126849	3-Deazauridine	Synthetic
164011	Rubidazone	Synthetic
177023	Levamisole	Synthetic
178248	Chlorozotocin	Synthetic

Publications by Staff

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DRUG SYNTHESIS SECTION

DRUG SYNTHESIS AND CHEMISTRY BRANCH

SCOPE

The function of the Staff of the Drug Synthesis Section, is to assist in the design and in the procurement of unique molecular species as potential antitumor agents. This work is coordinated with other facets of the Developmental Therapeutics Program including those of the natural product, toxicology and clinical areas.

The principle activities of the section are:

1. Assist in the acquisition and monitoring of contracts for the synthesis of compounds destined for evaluation in various biological systems.
2. Surveillance of the chemical, biochemical, biological and medical literature to uncover leads to potential antitumor agents.
3. Institute structure-activity studies to obtain new concepts of molecular modification for anticancer drug design.

STAFF

The personnel of the Synthesis Section consists of three professional members and one secretary. The professional staff are project officers for the contract activities and collaborate with the Branch Chief to fulfill the functions of the section.

SYNTHESIS ACTIVITIES

The objectives of the synthesis contractors are to provide the program with new chemical agents for anticancer evaluation. Their laboratories function to supply compounds of diverse structures and complexity.

Suggestions for syntheses which improve the effectiveness of anticancer agents may result from structure-activity studies of known compounds. The parent compound can be a new or novel structure, e.g., a synthetic compound which is an intermediate from a reaction sequence, a natural product, an important clinical agent, etc.

Syntheses also may originate from the imaginative designs of a scientist based on leads from the screening program, resolution of questions on biochemical mechanisms of action, biochemical or biological rationales and from the literature.

For convenience, the work of the synthesis contractors for the past year may be reviewed under the following headings:

I ANALOG DEVELOPMENT

- Nucleosides
- Anthracyclines
- Alkaloids
- Folic Acid
- Cyclophosphamide
- Metal Complexes
- Natural Product Fragments
- Central Nervous System Agents
- Triazenes and Miscellaneous Agents

II NEW LEAD DEVELOPMENT

I ANALOG DEVELOPMENT

Nucleosides

The design of fraudulent nucleosides and related heterocyclics as antimetabolites has provided one of the most important sources of anticancer drugs. At least six of these agents are in clinical use today. Present investigations are intended to develop agents which resist enzymatic degradation in tumor cells. The approach utilizes established and developing biochemical rationales to guide the design and synthesis of new agents. Synthesis at the University of Utah covers tricyclic nucleosides, C-nucleosides, and bicyclic nucleosides with unusual bases, e.g., deazapurines and pyrazolopyrimidines. Tricyclic nucleosides apparently are unknown in nature but show good and unusual selective anticancer activity. Other nucleosides of high interest for synthesis at Utah are those of "the transition and suicide" type.

The Southern Research Institute contract encompasses synthesis of both purine and pyrimidine-like nucleosides. Compounds of considerable interest are deaminase inhibitors, isonucleosides, a variety of nucleoside with the base attached at position other than the normal C-1 position of the sugar, 2'-deoxyribonucleosides and carbocyclic analogs. It is thought that 2'-deoxyribonucleosides may provide specificity for cancer cell DNA enzymes. After activation by kinases, some of these analogs may inhibit enzymes that effect interconversions of nucleotides. In initial tests, two very effective agents have resulted from this work, a 2-fluoropurine arabinoside and a carbocyclic analog of cytidine (NSC-266519).

Anthracyclines

Daunorubicin and adriamycin are clinically important cancer drugs. However, both compounds exhibit cardiac toxicity and myelosuppression. The molecule of both drugs contains the same aminosugar but differ in the aglycone to which the aminosugar is attached. In the search for more effective and less toxic analogs of these compounds, the synthetic approaches concern chemical modification of either the whole drug or modification of each of the constituent parts (sugar and aglycone), followed by coupling to give the final product. The analogs may provide better activity through a variety of actions including:

1. Different binding affinity toward different DNA's.

2. Enhanced rate of penetration and intracellular distribution.

3. Delayed metabolic inactivation.

Work on the intact drugs and the aglycone is contracted to Farmitalia and Stanford Research Institute. One important discovery by Stanford is that bridging of two molecules of adriamycin at position 13 results in a high level of antitumor activity. Work on the synthesis of aminosugars of different configurations is contracted to Ohio State University. Six of the required aminosugars and two deaminosugars of interest have been synthesized and quantities are being scaled up. These contractors also are involved in coupling studies. Over ninety agents have resulted from this project in 1976. Of these, eighteen compounds were selected for special screening and cardiotoxic studies. A matrix of structure-activity (antitumor and toxicity) is gradually taking shape and the redesign of improved drugs in this area is very promising.

An anthraquinone with a bis(hydroxyethylaminoethylamino) substituent has shown impressive activity in both the standard primary antitumor screens, P388 and L1210. This compound is structurally similar to the anthracyclines. Midwest Research Institute has synthesized eleven analogs of the anthraquinone and of those tested, two have shown good P388 activity (T/C 270 and T/C 168).

Alkaloids

Excellent progress has been made by the University of British Columbia contractor, during the past year. The main objective of this contract is to develop a versatile, practical synthesis of the dimeric vinca alkaloids, vincristine and vinblastine, and structurally related analogs. Dimerization reactions have been studied using the appropriately functionalized monomeric units, cleavamine and dihydroindole. Several dimeric alkaloid products were submitted for in vivo evaluation. The contractor has resolved the synthesis problem of introducing the correct stereochemistry in the coupling at the C-18 position of two alkaloid halves to provide the new dimeric indole alkaloids. Patent applications have been filed. A number of the contractor's synthetic derivatives have shown antitumor activity comparative to that of vinblastine and/or vincristine, but with markedly reduced toxicity. Two are being considered for clinical trials in Canada. The contractor has provided radiolabeled materials for NCI and has also aided in the structural elucidation of several metabolites.

Midwest Research Institute has prepared new analogs of the alkaloid ungeremine which show preliminary activity. The contractor made Coralyne in excellent yield by a five step practical synthesis and a number of analogs one of which is highly active (2 cures) in the murine P388 tumor. Work on analogs of the alkaloid streptonigrin has produced products with the AB and the CD ring structures. Studies are underway to determine if the spectrum of activity of the parent will be retained in related analogs. The structure-activity parameters of the nitidine group also are under investigation. Nitidine is a candidate for clinical trial.

Microbiological Associates successfully completed a ten step synthesis of 8-(3-aminopropyl) ethidium chloride. The compound has shown inhibition of foci formation of Simian Sarcoma Virus in rat kidney cells.

Folic Acid

Southern Research Institute, as a large multiple project contractor, is involved in synthesizing analogs of folic acid and methotrexate. The objectives are to synthesize compounds; a) with more favorable transport properties without appreciably lowering the capacity to inhibit dihydrofolic reductase; b) inhibitors of enzymatic systems other than dihydrofolic reductase; and c) compounds with greater specificity and less toxicity. The compounds synthesized include: modifications in the C_9-N_{10} bridge and the benzene ring; modifications in the pteridine ring, peptide and amide congeners involving the glutamate moiety, and alterations in the polar character of the side chain. The contractor undertook a study of the synthesis of citrovorum factor and supplied us with a kilogram of the pure material.

A study of the data of folic acid antagonists suggests that the more lipophilic the center linkage joining the aminopyrimidine terminal and the amino acid terminal, the better the antifolate activity. Midwest Research Institute, along with other projects in the synthesis area, is completing the synthesis of a folic acid analog with a quinazoline ring replacing the pteridine moiety and a methylene group replacing the nitrogen at position 10.

Cyclophosphamide

Cyclophosphamide, a clinically effective agent, is itself inactive and becomes effective only after appropriate activation in vivo. Some effort has been devoted to the elucidation of the chemistry of metabolic activation and to the synthesis of analogs of the active metabolite, i.e., phosphoramide mustard. Aldophosphamide, the key intermediate in the cyclophosphamide metabolism, and a series of analogs of aldophosphamide have been prepared. Additional examples of phosphoramide mustard and its analogs have been synthesized and submitted for testing. The work in this area was performed by Collaborative Research, Inc.

Metal Complexes

The transition metal coordination compound area provides a rich source of antineoplastic agents. The search continues to find a replacement for the useful but relatively water insoluble and quite toxic clinical agent cis-diamminedichloroplatinum (II). In the past year a number of new highly active, water soluble platinum complexes were produced by the contractor, Veteran's Administration Hospital. One of these (NSC-271674 soluble as the sodium salt), in preliminary testing produced five cures out of ten in mouse L1210 leukemia and is in the process of being patented. A number of platinum (IV) coordination compounds were synthesized and exhibit greater water solubility and as much activity as the corresponding platinum (II) complexes. The contractor has synthesized active complexes of other transition metals, for example, rhodium. Another objective has been to find new classes of active complexes; those that are both water soluble and that may be eliminated via the biliary rather than the renal route. The Project Officer has reviewed the data of 626 platinum complexes and disclosed his conclusions to the Principal Investigator. Among these, the most interesting is that the activity of a complex appears to be insensitive to electronic effects by substitution on aromatic ligands and that configuration is an important contributor to the activity of the most active compounds.

Several groups are interested in carrying out clinical trials of the most promising of these new agents. Unfortunately, development of a suitable toxicity test to measure renal toxicity has been beset with difficulties. Selection of highly active, water soluble, platinum compounds still awaits toxicity testing for their possible introduction into the clinic.

Natural Product Fragments

Bleomycin, an effective but structurally complex compound, is used both as antibiotic and anticancer agent. Investigation shows that the molecule may be divided into five unique amino acid fragments. The synthesis of several key intermediates in the construction of three of the five fragments is complete. The contractor, Massachusetts Institute of Technology has succeeded in preparing a blocked pseudodipeptide as the immediate precursor of Fragment I (β-lactam of 4-pyrimidinecarboxylic acid-2-β-alanine). Studies on the synthesis of Fragment II (disaccharide derivative of histidine) continues. Progress has been made in the preparation of Fragment III (dipeptide of homoserine and 4-amino-3-hydroxy-2-methylvaleric acid), while the synthesis of Fragment IV [2-(2-aminoethyl-4-thiazolyl)-4-thiazolecarboxylic acid], was achieved. Interestingly, some of the intermediates have shown anticancer activity. The synthesis of analogs of the active intermediates is in progress and will be the main thrust in the coming year.

Central Nervous System Agents

The development of CNS-penetrating antineoplastic agents is of importance for the treatment of primary CNS neoplasms and for destroying lesions which are metastatic to the brain. A basic limitation to CNS chemotherapy has been the synthesis of drugs which will both penetrate the CNS and retard the tumor's replication. The development of agents with these characteristics has involved the synthesis of lipid soluble compounds with antineoplastic activity. Such compounds should have the potential to localize specifically in the brain. Northeastern University has synthesized two classes of compounds as potential CNS antineoplastic agents; aporphine mustards and 3-aminoisoquinoline mustards. The alkylating agent was chosen as the antineoplastic moiety. More than fifty compounds have been prepared and tested intraperitoneally in the L1210 system. Of these, two have shown preliminary activity. Additional studies are scheduled in the ependymoblastoma and other brain tumor systems.

Triazenes and Miscellaneous

Another active area of research at Southern Research Institute is the synthesis of certain chemically reactive types of compounds as potential anticancer agents. Triazenes and hydrazines, analogs related to dicarbazine and procarbazine were synthesized. These compounds may be active *in vivo* by microsomal oxidases and may demonstrate anticancer activity. Work on nitrosoureas has been terminated with the exception of a few selected compounds as these areas are being deemphasized.

At Stanford Research Institute some of the effort is devoted to anthracyclines (see section on anthracyclines), methotrexate studies, benzo [i]-phenanthridine and ellipticine intercalators, mesoionic 1,2,3,4-oxatriazole, glyoxylic acid tosylhydrazone, and piperazinedione. About 10% of the work is on the resynthesis of active compounds for additional studies. Two oxatriazoles and one tosylhydrazone have been selected for testing by the new antitumor panel.

A contract with the Central Drug Research Institute, Lucknow, India, has as main objectives: (1) synthetic development of potential anticancer agents, (2) the collection of indigenous plants and isolation of active materials for correlative chemical, biochemical or botanical studies, and (3) establishment of a screening unit to bioassay plant products and the synthetic compounds produced. The plant collection and isolations have continued to provide the program with numerous extracts. There are three approaches to the synthetic part of the program; the modification of natural product molecules found to possess anticancer activity, the synthesis of nitro aromatics and heterocyclics, and the synthesis of compounds related to the structural unit in emetine. Two compounds which incorporate the pharmacophore of emetine, a structural unit associated with inhibition of protein synthesis were made. These are 4-(2-piperidinylmethyl)-1-methylpiperidine and 6,7-dimethoxy-1-(3-ethyl-1-methyl-4-piperidinyl)-1,2,3,4-tetrahydroisoquinoline.

Six seco C-ring modifications of ellipticine, ten analogs of arnebin, a large number of simple amides of actinomycin, and five analogs of aristolochnic acid were synthesized. One of the actinomycin amides is active in the P388 murine tumor system. Testing data is not yet available on all of these products.

II NEW LEAD DEVELOPMENT

One of two recently acquired contracts, the contract at the University of Southern California, has as its objective the synthesis of (a) cyclopentenoisoquinoline derivatives of minimum complexity to bond specifically to guanine-cytosine pairs across the double helix of DNA, (b) a new "K_{cat}" inhibitor of dihydroorotate dehydrogenase, and (c) 5-ethynyluracil and its ribo and deoxyribonucleosides. Initial attempts at fulfilling these objectives appear to be on schedule.

St. Jude Children's Research Hospital seeks to make inhibitors of pyrimidine utilizing enzymes postulated to predominate in certain types of tumor cells. These cells acquire their needed pyrimidines through a salvage pathway and are unable to synthesize the compounds *de novo* as do normal cells.

Inhibition of the pyrimidines should result in cell death or retard the growth of the tumor cells. The inhibitors are analogs of pyrimidine nucleosides, e.g., 1-deazapyrazofurin, 2-deazapyrazofurin, their corresponding nucleosides and 1,2,4-thiadiazole ribofuranoside.

In addition to the work reported under alkaloid, folic acid, and anthracyclines, Midwest Research Institute is synthesizing alkoxybenzo[g]quinones and 7,8-benzochromones. This effort is based on the reported activity of these types of compounds and material requested by NCI staff.

The synthesis areas of Microbiological Associates include exploration to determine the anticancer potential of novel structures of alkaloids, antibiotics, aminoacids, and polyamines. The selection of projects is based in part on the quality of the investigator's rationale and the rapidity with which the synthesis can be implemented to provide products for evaluation. Thus a large promising area of potential antitumor agents for investigation is surveyed and any newly discovered active structural moieties exploited. The work on ethidium analogs was reported under the alkaloid section. The contractor has made a number of in vivo active alanosine analogs upon request from NCI staff. The studies on bisquinaldine intercalators provided a series of active compounds which are being further evaluated. The contractor has in testing two unique compounds especially designed for tumor cell surface interaction. In one, a peptide is tied to a ferrocene moiety through an alkyl chain. In the second, a polyamine chain replaces the peptide unit. It is postulated that these types of compounds through binding to DNA on tumor cell surfaces will enhance the immune response resulting in tumor destruction.

The Section has responsibilities for twenty-two synthesis contracts as shown on Table VI.

TABLE VI

CONTRACT LABORATORIES FOR SYNTHESIS

Contractor	Investigator	Contract No.
British Columbia, Univ.of	Kutney	N01-CM-23223
Central Drug Research Institute	Dhar	PL-4-8001
Collaborative Research, Inc.	Friedman	N01-CM-6056 and
Collaborative Research, Inc.	Friedman	N01-CM-12326*
Colorado, University of	Hassner	N01-CM-43779*
Dow Chemical Company	McCarthy	N01-CM-33724*
Farmitalia	Bernardi	N01-CM-57036
Farmitalia	Arcamone	N01-CM-57014
Illinois, University of	Rinehart	N01-CM-23208*
Massachusetts Institute of Technology	Hecht	N01-CM-43712
Microbiological Associates, Inc.	Dubois	N01-CM-43761
Midwest Research Institute	Cheng	N01-CM-33743
Northeastern University	Soloway/Neumeyer	N01-CM-53741
Ohio State University	Horton	N01-CM-57015
Pennwalt Corporation	Block	N01-CM-12309*
Rhode Island, University of	Goodman/Abushanab	N01-CM-43778*
St.Jude Children's Research Hospital	Welch	N01-CM-77127
Southern California, University of	Heidelberger	N01-CM-67084
Southern Research Institute	Montgomery	N01-CM-43762
Stanford Research Institute	Henry	N01-CM-33742
Utah, University of	Townsend	N01-CM-43806
Veteran's Administration Hospital	Gale	Y01-CM-60102

*Contract terminated during this report period.

CHEMICAL AND DRUG INFORMATION SECTION

DRUG SYNTHESIS AND CHEMISTRY BRANCH

Scope

The Chemical and Drug Information Section is responsible for: (1) operating, maintaining, and enhancing the automated Drug Synthesis and Chemistry's Chemical Information System (CIS) which supports the accessioning storage, and distribution of chemicals and drugs acquired for antitumor testing, including the coordination of staff and contract activities related to the CIS: (2) managing the storage and retrieval of chemical information in both automated and manual DTP files; (3) conducting structure/activity studies to optimize acquisitions, by identifying chemical classes to be deemphasized because they are largely negative or adequately studied and by developing models for assigning to potential acquisitions, activity scores in the P388 lymphocytic leukemia prescreen; and (4) identifying for acquisition compounds of interest which are reported in the literature or are in various chemical files maintained by governmental agencies, industrial organizations, and information venders.

Staff

The Section's staff consists of two chemists, two system analysts, one mathemetician, one chemical information clerk, and three supporting clerks.

Emphasis of Effort

During this report period, the Section concentrated on: (1) improving the operation of the CIS and implementing various enhancements which had been deferred to expedite the startup of the shipping and inventory subsystem; (2) providing chemical information to DS&CB and other DTP decision-makers; (3) experimenting with statistical and pattern recognition methods in correlating chemical fragments with biological activity to develop a model for predicting antitumor activity in potential accessions; (4) completing the feasibility study of systematically monitoring the literature to identify compounds with reported biological characteristics which would warrant acquisition for antitumor testing; and (5) reviewing the system documentation effort by the CIS implementation contractor (University of Pennsylvania) to assure the transfer of knowledge to the CIS operations contractor (Chemical Abstracts Service).

Chemical Information System (CIS)

This automated system enables a limited in-house staff to monitor the accessioning, storage, and distribution of chemicals and drugs acquired for antitumor testing. In processing acquisitions, the system identifies duplicates and analogs of Program interest, produces action and informational documents required by the staff and contractors involved with accessions, maintains inventory control, and tracks acquisitions from their receipt through shipment. In addition to accelerating the accessioning process, the CIS provides improved operational control in monitoring the contractors engaged in this activity. In March, 1977, the system stored and permitted searching information on 300,000 compounds. An index of names is generated and maintained to determine whether a named compound is in the DS&CB collection or has been reported in Chemical Abstracts thereby facilitating access to the literature.

After the shipping and inventory subsystem was installed in Spring, 1976, changes were made to the CIS in response to operational needs. Typical enhancements include improved reports to system users, the automated transfer of additional information to the Biology System, and the addition of new data to the automated file, such Chemical Abstracts Registry Numbers. An audit of the files which contains some 350,000,000 bytes (characters) of information demonstrated few errors despite the complex interrelation of data in the file, and the less than optimal automation of file corrections.

The Special Structure Feature (SSF) module of the CIS was designed to identify compounds which possess structural characteristics of Program interest. Such compounds are given special handling rather than automatically shipped for routine prescreening. The installation of the processor in the Summer, 1977 will permit acquisitions to be automatically searched against a large number of chemical features of Program interest and will be useful in the selective acquisition and testing of chemicals.

The University of Pennsylvania has implemented and installed the CIS at Chemical Abstracts Service (CAS) which operates the system under contract. The University of Pennsylvania has delivered the programming documentation and has trained the CAS programmers to assume system maintenance responsibility. The remaining task of the University of Pennsylvania is to update a comprehensive INQUIRY Users' System Manual which will serve as a reference tool for DS&CB and CAS staff.

Automated Retrieval of Information

INQUIRY, the on-line chemical search system, has been used extensively to meet the chemical information needs of investigators. Terminals allowing access to structural and non-structural data are linked via a long distance line to an IBM 370-168 computer at Chemical Abstracts Service in Columbus, Ohio. The query capability is available to staff and to authorized staff of contractors involved with the acquisition processing activities, i.e., the acquisition, data processing and storage and distribution contractors.

The search system is used routinely to provide an immediate answer to the question "Does the compound collection include chemical X?" This question must be answered before assigning an accession (NSC) number to a compound that must be shipped for testing on a priority basis. Queries of this nature also arise frequently within DCT for other reasons or are posed by outside investigators. (Queries regarding named compounds are answered often by consulting secondary references such as the Drug Development Index of non-systematic names or by searching on-line the National Library of Medicine's CHEMLINE system.) Routine searches are also made to answer operational questions dealing with suppliers, shipments, inventory, etc. The more critical use of the query capability, however, was to conduct from July, 1976 to April, 1977 the more than 170 substructure searches for analogs of Program Interest.

These queries were requested by the Data Review Subcommittee, the Drug Evaluation Committee, individual staff members, and investigators outside DCT. Searches were made for the over 3,600 nucleosides in the Program for use by the coordinator of the nucleoside analog study group. Similar information was provided on metals for the platinum study group and for the anthracycline study group. Extensive analog searches were made to identify negative and adequately studied chemical classes which were recommended for deemphasis in furture acquisitions.

The search system was studied to reduce both the time spent by the user on the terminal and computer costs. Cost-effective changes are being implemented which will enable the one terminal used by staff to continue to meet the ever-increasing chemical information retrieval requirements. Within funding constraints, the most significant improvements of some other 40-odd desireable changes are being implemented to increase search effectiveness. Reduced terminal usage and lower computational demands per query is imperative to avoid the need of a second terminal by staff. The size of the INQUIRY system and the concurrent use of three terminals (at DCT, CAS, and the storage and distribution contractor) place a heavy load on CAS' computational resources.

Structure/Activity Studies

The computerized chemical and biological data bases are being analyzed to develop acquisition policies and automated mechanisms for optimizing the selection of compounds for antitumor testing. As significant milestones are reached, results are presented to the Preselection Committee which was established to optimize acquisitions.

Chemical Classes Deemphasized. (See Table VII) Three potential negative classes were investigated namely: (1) hydrocarbons, excluding compounds with three or more aromatic rings; (2) halogenated hydrocarbons; and (3) simple compounds with twelve carbons or less, with one selected functional group or two identical functional groups. Substructure searches of the DS&CB file were made to test these criteria. Hydrocarbons containing only carbon and hydrogen were largely negative, displaying a ratio of activity less than one third of the average activity of the entire collection. Halogenated hydrocarbons, however, were slightly more active than the average activity of the entire collection. Simple compounds with twelve carbons or less (See Tables VII and VIII) were significantly less active than the average activity of the entire collection except for compounds having the following functional groups: epoxides, hydrazides, hydroxamic acids, azides, cyanides, ureas, hydrazines, nitro, sulfoxides, and sulfonic acid esters. A study of potential adequately studied classes (See Table IX and the accompaying text) identified two additional negative classes: sulfa drugs (*p*-amino benzenesulfonamides and barbituric acids. However, all of these negative classes constituted only 7.7% of the total compounds in the collection and appear infrequently in recent acquisitions. Additional experimentation in this area is needed.

TABLE VII

CHEMICAL CLASSES DEEMPHASIZED

	NCI ACTIVE	NCI TESTED	NORMALIZED ACTIVITY RATIO(2)
Hydrocarbons(1)	10	1614	.29
Halogenated Hydrocarbons(1)	53	2102	1.0
Simple Compounds with 12 Carbons or less	75	9845	.34

(1) Excluding compounds with four or more aromatic rings.

(2) Normalized activity ratio = $\frac{\text{actives in a class}}{\text{all compounds in a class}} \div \frac{\text{all actives}}{\text{all compounds}}$

Ratio of 1.0 equals the average activity of the entire collection.
Ratio $>$ 1.0 equals activity greater than average.
Ratio $<$ 1.0 equals activity lower than average.

TABLE VIII

ANTITUMOR ACTIVITY IN COMPOUNDS WITH TWELVE OR LESS CARBONS

Functional	Actives	Total Collected	Normalized Activity Ratio(1)
Acyl Halides	2	120	.8
Aldehydes	2	133	.65
Amides	8	893	.38
Amidines, Imines, Oximes	0	228	0
Amines	8	928	.37
Azides(2)	1	17	2.3
Carbamates	2	408	.21
Carboxylic Acids	0	701	0
Cyanides(2)	3	135	.96
Epoxides(2)	7	33	9.2
Esters or Lactones	3	1278	.10
Ethers	2	266	.32
Hydrazides(2)	7	151	2.0
Hydrazines(2)	1	42	1.03
Hydroxamic Acids(2)	2	38	2.3

(Table VIII continued next page)

TABLE VIII (continued)

ANTITUMOR ACTIVITY IN COMPOUNDS WITH TWELVE OR LESS CARBONS

Functional	Actives	Total Collected	Normalized Activity Ratio(1)
Hydroxy	1	2296	.04
Ketones	1	463	.09
Mercaptans	0	22	0
Nitro(2)	5	167	1.3
Phosphorous-Carbon & Oxygen containing	1	434	.10
Quinones	1	65	0
Semicarbazides, Azoxy	0	225	0
Sulfides	0	85	0
Sulfones	1	100	.43
Sulfonic Acid Derivatives	1	109	.39
Sulfonic Acid Esters(2)	8	90	4.0
Sulfoxides	4	145	1.2
Ureas(2)	5	224	.97
TOTAL	75	9793	.34
TOTAL, excluding functional groups with > average activity of the collection.	32	8803	.16

(1) Normalized activity ratio = $\frac{\text{actives in a class}}{\text{all compounds in a class}} \div \frac{\text{all actives}}{\text{all compounds}}$

> (2) Functional groups with > average activity of the entire collection.

Adequately Studied Classes. When a class of compounds has been studied in depth, there is a decreased probability of that class showing novelty. Chemical classes heavily represented in the collection were searched and analyzed to specify the total number of compounds and the number of actives in each class. (See Table IX) The following classes were recognized as being Bbβα adequately studied: bis-(β-chlorethyl) amino, i.e., nitrogen mustard; bis methanesulfonates; mono-α or β-haloacyl derivatives; and 6-mercaptopurine and thioguanine derivatives which differ from the parent only in the substituents at the 9-position and/or by substitution on sulfur. These adequately studied subclasses constituted 2.5% of the sample collection. A detailed study classifying and analyzing the activity of subclasses of purines (4,500 compounds) and pyrimidines (8,500 compounds) has been completed and recommendations made to the preselection subcommittee. Tentatively, it appears that the following classes may also be adequately studied: hypoxanthins;

caffeines; 2-amino 4-oxy pyrimidines; and 2-amino 4,6, (not N,O,P,S) pyrimidines; and 2,4,6 (C or H only) pyrimidines (simple derivatives). These additional classes increase to 3.6% the proportion of adequately studied compounds to the total collection. Other large classes such as carbamates are being categorized into subclasses useful for guiding acquisition efforts.

TABLE IX

ADEQUATELY STUDIED CLASSES

Class	Actives	Total Collected	Total Reported Literature(1)
Barbituric Acids (dialkyl)	2	462	2000
Bis Methanesulfonates	76	550	1500
Bis or Tris Aziridines	206	432	2200
6-Mercaptopurine	192	860	2500
Mono-α or β-Haloacyl	75	2250	7300
Nitrogen Mustards	707	2708	7300
Purine	299	4500	27000
Pyrimidine	236	8500	57000
Sulfa Drugs (*p*-amino benzenesulfonamides	5	1221	7900
6-Thioguanine (not included in 6-MP)	169	574	800

(1) Searched on test file of 275,000 compounds reported by Chemical Abstracts Service (CAS) and projected to the total CAS file of 3.4 million.

Probability-based Preselection Model. Statistics on structure-activity relations over broad classes of structures are being used to develop a model to predict the probability of antitumor activity of potential acquisitions in the lymphocytic leukemia (P388) prescreen. Theoretically this concept could be applied to any set of biological data. The immediate objective of this effort is to use the model in the Chemical Information System environment for the acquisition of promising compounds. An estimate of the probability of activity would be brought to the attention of a decision-maker as well as a measure of rarity or uniqueness, i.e., the occurence of structural fragments infrequently present in DS&CB compounds.

Predictions were made on a test set of 2,322 compounds having definitive test results. These compounds were not included in the training set used to develop the model. The test demonstrated that if 50% of the compounds had been selected for testing (i.e., the half with the higher predicted values for probability of activity, only six out of 111 actives would have been lost and the percentage of active compounds would have been enriched from 4.8 to 9.1%. (See Table X)

TABLE X

SEPARATION OF ACTIVE COMPOUNDS

BY THE P388 LYMPHOCYTIC LEUKEMIA PRESELECTION MODEL

Calculated Activity by Deciles	Number of Compounds with %T/C ≥175	Number of Compounds with %T/C >120 <175
Highest	17	45
Ninth	6	16
Eighth	0	12
Seventh	2	2
Sixth	1	4
Fifth	0	1
Fourth	0	4
Third	0	0
Second	0	1
Lowest	0	0
TOTAL	26	85

The structural fragments in both the training and test sets are being analyzed with the view of modifying the model to reduce false negatives. Another set of 4,000 compounds screened in P388 will be tested by the model to determine its reliability. During the Summer, 1977 we will test the effectiveness of the model in aiding the acquisition contractor's staff to select compounds with a higher than historical probability of activity in the P388 prescreen and to identify compounds possessing structural fragments not present or rarely found in the collection.

Literature Surveillance

The Section completed a literature surveillance feasibility study, using primarily the Chemical Abstracts data base. This study demonstrated the potential of using a variety of automated data bases for identifying compounds with biological activities of Program interest. The staff is reviewing the search criteria by investigating the correlation of various biological activities with antitumor activity and estimating the potential yield of such compounds from the literature and from other collections. The major objective is to acquire compounds with structural characteristics unique to our file and possessing biological activities of interest to the Program for testing in the DCT panel of screens. Compounds identified by this method as being worthy of prescreening would also be acquired.

Linking the Biological and Chemical Data Bases

In joint participation with the Automated Information Section of the Drug Evaluation Branch, this Section is studying the feasibility of linking the biological and Chemical Information System to provide more rapidly and in an optimal format information from both data bases. This capability will more effectively support structure activity relationships studies, the work of the DTP decision-making committee, and provide improved capabilities in a variety of other activities, such as publication.

Plans For the Coming Year

Chemical Information System. A variety of tasks will increase the effectiveness of the system's response to information needs. The capability of displaying structural images, including stereospecific information, for all active compounds will be provided. OLSIS, the on-line structure input system, which was developed with CAS funds, will be used operationally to input structures of new accessions which possess stereospecific information. GDS, a program which generates the display of the structure from the computerized record of structures, will be integrated into the system and display structures for 85 to 90% of the compounds collected. (These planned enhancements to the system are basic to implementing an interlink between the biological and chemical information systems.) The effectiveness and efficiency of the search system will be improved by: facilitating the formulation of queries; retrieving structural information now not made available; and improving the response time and the efficiency of machine resource utilization. The file correction capability will be automated to the extent feasible to assure the continued integrity of the data base. A variety of programming changes will be made to improve operational effectiveness of the system related to the processing of acquisitions.

File Analysis. Within the limits of staff resources, the analysis of the chemical collection will continue to identify additional negative and adequately studied classes to be deemphasized in acquisitions.

Integration of the Preselection Model into the Chemical Information System. The improved Search on Topology program which matches potential acquisitions against the file to identify duplicates and near-duplicates will be used more extensively. The Special Structural Feature identification program module will be used operationally to identify analogs of Program interest and will be expanded experimentally to include search profiles to identify compounds in negative and adequately-studied chemical classes. The P388 preselection model will be used experimentally on potential acquisitions to simulate an operational mode. These automated mechanisms, each serving as "stand-alone" tools need to be integrated into the Chemical Information System so as to examine the structure of a potential or actual acquisition, compare the structure with the chemical and biological information in the file, and report compounds which are not known analogs but which have a high probability of activity in the P388 prescreen. This integrated approach will be simulated within existing resources.

Other Tasks. If the biological and chemical data bases are to be linked, this Section will participate heavily in implementing this capability. Continued support will be provided to the literature surveillance effort.

TABLE XI

CONTRACTS MONITORED BY THE SECTION

Contractor	Principal Investigator	Contract No.
Chemical Abstracts Service	Dunn	NO1-CM-43722
University of Pennsylvania	Lefkovitz	NO1-CM-33719

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM-07101-02 DSCB

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)
Computer Methods for Drug Preselection Based on Structure-Activity

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	L. Hodes	Research Mathematician	DS&CB, NCI
OTHER:	G. Hazard	Chemist	DS&CB, NCI
	S. Richman	Head, Chem. & Drug Info. Sect.	DS&CB, NCI
	R. Geran	Biologist	DEB, NCI

COOPERATING UNITS (if any)
Chemical Abstracts Service

LAB/BRANCH
Drug Synthesis and Chemistry Branch

SECTION
Chemical and Drug Information Section

INSTITUTE AND LOCATION
NCI, NIH, Silver Spring, Maryland 20910

TOTAL MANYEARS: 1.2	PROFESSIONAL: 1.2	OTHER: 0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This study was initiated to provide information about chemical structures as an aid in selecting from potential acquisitions, compounds for screening. Statistics on structure-activity relations over broad classes of structures are being used to predict the probability of antitumor activity of new compounds in the mouse lymphocytic leukemia (P388) screen. Also, a measure of rarity or uniqueness of a new structure is derived from the statistics on the structural characteristics of compounds that have been adequately screened in P388, regardless of their activity. It is planned to integrate the P388 model into the DTP Chemical Information System for use operationally to aid in the selection of compounds for screening.

PHS-6040
(Rev. 10-76)

Objectives:

1. Evaluate the potential of structure-activity determinations over broad ranges of compounds for specific biological test systems.

2. Demonstrate the feasibility of such methods to preselect compounds for antitumor screening, e.g., in setting priorities or in selecting appropriate test systems for screening.

3. Within the screening capacity, maximize the acquisition of additional promising compounds or compounds inadequately represented in the file.

4. Install these methods into the Chemical Information System operating environment.

Methods Employed

1. The method for predicting the probability of activity uses known biologically active and inactive compounds as training sets in such a manner that the incidence of molecular fragment keys in these sets allows for estimating a measure of the probability of activity of new compounds containing some of these fragments. This method is described in the publication cited below.

2. Molecular structure fragments that were previously developed as keys for querying the automated DTP Chemical File are being employed as predictor variables. Another set of molecular structure fragments developed for searching the Walter Reed Army Institute of Research collection were tried.

3. Biological data, now limited to the P388 screen, is obtained from a query of the DTP Biology File. The criterion for activity is a confirmed increase in the life span of 20%. Active compounds with a confirmed increase lifespan of 75% or more are kept separately. Also obtained was a set of negative compounds i.e., those which were tested adequately and failed to increase the lifespan by 20% or more.

4. To get a measure of the rarity or uniqueness of new compounds, a list was compiled of all the keys appearing in P388 tested compounds and their incidence. When a new compound enters, its least frequent key is printed together with its incidence. If the compound has a key which did not occur on the list, this unique key is reported.

Major Findings

1. Several experimental results supported the usefulness of the method on the P388 prescreen. A large scale experiment used a set of 2,716 active compounds as the training set. Scores derived from the set of 15,524 inactives did not improve the performance in the P388 model. Prediction was done on a set of 2,322 compounds tested in P388 subsequent to the

training set. The result showed that if we had preselected for screening 50% of the compounds (i.e., the half with the highest predicted value for probability of activity) then six actives out of 111 would have been lost. Thus the percentage of active compounds would have been enriched from 4.8% to 9.1%.

2. Since this is a statistical method, its performance on the training set itself is not guaranteed to be perfect. Out of the 838 compounds in the training set with a confirmed increase in life span of at least 75%, 21 compounds fell below the 50% mark of the test set described in 1. above. This is a 2.5% false negative rate for highly active compounds.

3. A frequency distribution of the molecular fragment keys of the 21,512 compounds included in the training set reported a total of 5,145 different structure keys. Applying the uniqueness measure to a set of 5,000 compounds excluded from the training set provided the following results: 5% of the compounds had a unique key; and 30% of the compounds had keys which were in the training set with a frequency less than 0.1%.

4. A set of structure keys developed at Walter Reed Army Institute of Research (WRAIR) along lines radically different from the NCI keys was tested on the small data set described in last year's report. Although the keys had desirable features, they did not work nearly as well as the NCI keys. This is believed due to their lack of specificity in bond and ring system information.

Significance for Biomedical Research and the Program of the Institute

1. The enrichment effect produced by preselecting compounds should increase the number of actives within the current screening level and maximize the potential of screening compounds with unique structural characteristics not adequately represented in the NCI collection.

2. The method developed for the prediction of probability of activity is now recognized as a contribution to the state of the art of broad range structure-activity relation work. It can be applied to many areas, e.g., using carcinogenicity or toxicity as the biological activity.

Proposed Course

1. The favorable results call for an early integration of the P388 preselection model on an experimental basis into the acquisition routine. This involves a change in the system to permit generation of keys before a compound is registered. A report on the estimate of the probability of activity and the presence of rare keys in candiidate acquisitions would be generated. This information is not intended to determine the selection of acquisitions but rather to serve as decision-making aids.

2. New keys are being investigated to improve performance of the model with respect to false negatives. Also planned are approaches to generating new keys based on broad classes as well as distinguished subsets of chemicals.

3. An experiment is under way to remove from the model classes of compounds which have been adequately studied. These compounds comprise about one third of the actives in the training set. This revised model may improve the prediction of activity of compounds in less familiar chemical classes. Analogs of known actives would be identified by another program module of the Chemical Information System.

Publications

Hodes, L., Hazard, G. F., Geran, R., and Richman, S.: A Statistical-Heuristic Method for Automated Selection of Drugs for Screening. J. Med. Chem., 20: 469-475, 1977.

ANNUAL REPORT
NATURAL PRODUCTS BRANCH
DEVELOPMENTAL THERAPEUTICS PROGRAM
DIVISION OF CANCER TREATMENT
NATIONAL CANCER INSTITUTE

One of the major objectives of the Division of Cancer Treatment is to discover novel types of compounds with anticancer activity which can provide a basis for new chemotherapeutic agents.

In this regard natural product research has a long history of producing novel and unusual types of chemical structures which show many types of biological activity and indeed the basic structural types of various classes of natural products have led to development of many major classes of chemotherapeutic compounds. The investigation of natural substances for anticancer activity can provide new types of compounds for evaluation which possess unusual properties and can lead to new drug classes for chemotherapeutic evaluation in cancer treatment.

The Natural Products Branch has actively pursued acquisition, isolation, structure determination and testing of compounds from microbial, plant, and animal sources in order to obtain new leads for further development in the NCI program.

The major program areas of the Natural Products Branch include: 1) contract research directed towards isolation of new agents from active extracts of plant, animal (including marine organisms) and microbial sources; 2) worldwide procurement of new plants, marine organisms, and microbes for preparation of extracts and screening for anticancer activity; 3) procurement and preparation of large quantities of active agents for toxicological and clinical studies; 4) worldwide literature surveillance for the acquisition of natural products with demonstrated biological activity or novel structural types for evaluation.

Objectives

As part of the overall Division of Cancer Treatment Program, the Natural Products Branch has three major objectives:

I. Plant and Animal Products

The acquisition of new active antineoplastic agents from higher plants and from animal products (marine primarily) is the major function of this section. A contract program exists to obtain, select, and process enough plant material to obtain sufficient novel material for chemical identification and antineoplastic testing. Critical to this program is the ability to ensure a supply of plant material which is deemed of interest to NCI.

A contract program exists to obtain marine animal products in sufficient quantity to extract, isolate, and identify the active antineoplastic compounds.

II. Fermentation

The acquisition of new antineoplastic agents from microbial fermentations is the primary function of this area. A contract program exists to isolate unique organisms and to systematically evaluate the microbial world for its potential to produce antineoplastic agents. The capacity exists in these contracts to produce sufficient amounts of material for clinical studies.

III. Worldwide Surveillance of Natural Products

This area of work is carried out by the in house staff. Literature, personal contacts with scientists, universities and industrial companies allows NCI to determine if any new natural products with various biological activities have been isolated and are worthy of testing in the National Cancer Program.

Program Implementation

The objectives of the Natural Products Branch are carried out through the Sections and the Office of the Chief and are:

I. Plant & Animal Products Section

The objective is the development of novel anticancer drugs from higher plants and animals (including marine organisms and insects). In support of this activity the Section maintains a worldwide program of acquisition of plant and animal materials. These materials are supplied by two collection contracts, purchase of new extracts and voluntary submission from natural products researchers. A contract also exists to prepare extracts of new materials for preliminary screening. Materials corresponding to active extracts are then recollected in quantities large enough for fractionation studies and fractionation is undertaken by one of six contractors who undertake the isolation, purification, and structural elucidation of the active substances in the extracts. There are four contractors involved in isolation of novel antineoplastic agents from plant materials and two contractors primarily involved with animal extracts. These contractors also reisolate larger quantities of pure compounds for further screening as required. A preparative scale isolation laboratory is available to produce large amounts of pure compounds for toxicology and clinical studies.

The Section also works closely with scientists worldwide who are doing isolation studies of plant and animal materials. This work includes arranging for testing of samples, monitoring progress of isolation studies and lending assistance in natural products research in the anticancer area as needed. Their non-contract research is funded by NIH grants, PL 480 funds, or grants from universities or foreign governments.

II. Fermentation Section

The objective is the isolation and development of novel antineoplastic drugs derived from microbial fermentations. In support of these tasks, the Section maintains seven contracts to isolate unique organisms, and to systematically evaluate the microbial world for its ability to produce novel antineoplastic agents. The Section maintains four contracts to isolate, identify, and scale-up active materials of interest for NCI evaluation. These antineoplastic agents are supplied in quantities sufficient for toxicological and clinical trials.

The Section directs programs in biotransformation, co-metabolism and in the production of interferon from lymphoblasts. This work includes fermentation, development, and chemical isolation and genetic and culture research. More than 5,000 organisms are fermented/yr. and evaluated for their ability to produce novel antineoplastic agents. One contract is devoted to biotransformation of known structures in order to increase the activity, lower toxicity, or in general, improve the basic structure as an antitumor agent.

III. Worldwide Surveillance

This activity is carried out by the Branch and is important in the obtaining of novel natural products for evaluation in the program. The maintenance of worldwide contacts with scientists or industrial companies and universities is essential to obtain as early as possible any novel compounds with biological activity. These compounds are reviewed for interest and if deemed interesting are tested in the NCI program.

Branch Staff

The Natural Products Branch is composed of a staff of six persons consisting of two senior professional scientists (GS-13 or above), one other professional staff member and three technical and clerical staff. One additional senior staff position is vacant.

The responsibility for the activity of the Branch is as follows:

Natural Products Branch
Chief - Dr. John D. Douros
Plant and Animal Products Section
Head - Dr. Matthew Suffness

Fermentation Section
Head - Vacant

Program Operation

Fermentation Section

The fermentation program has been markedly changed during the last two years to hopefully obtain more novel organisms which will produce more novel antineoplastic agents. In order to cut costs in the in vivo testing of primary fermentation broths, more use has been made of pre-screens to select fermentations for in vivo testing. Although not ideal, the pre-screens used are Infectious Bronchitis virus test (1 contractor), cell cytotoxicity (KB, LE, PS by 3 contractors), and enzyme inhibition (1 contractor). The results of using a pre-screen allowed NCI to decrease the number of primary fermentations to be tested in vivo from 5,000 to 763 with an overall increase in the number of actives (due to different organisms being isolated). The fermentation program has made a marked effort to obtain novel organisms never before screened for their antineoplastic activity by using unusual substrates (hydrocarbons, terpenes, polyhydroxy acids, etc.) and varied culture isolation techniques (sprinkle plate, percolation, soil feeding, etc.). Results shown in Table 1 indicate that it was a highly productive year in obtaining new antineoplastic agents. However, the real benefits of the program will not be known until one sees how many of the new compounds are clinical candidates.

TABLE I

1976 FERMENTATION CONTRACT PROGRAM RESULTS

Contractor	Cultures Isolated	Pre-Screens Active In Vivo	Active In Vivo (PS leukemia) (a)	Undergoing Chemical Fractionation	Novel Antineoplastic Agents Isolated
A	2,158	319	64	15	9
B	846	88	22	6	3
C	1,425	196	38	35	14
D	534	160	38	18	4
	4,963	763(15.3%)	162(21.1%)	74	30

(a) Normally only 1% of the cultures isolated produce in vivo active fermentation broths.

Biotransformation and cometabolism are two techniques also being evaluated for their potential to produce interesting microbial metabolites. Several contractors are systematically evaluating the microbial world to attempt to identify which microbial type is most productive in producing novel antineoplastic agents. Biotransformation and the systematic evaluation of microbes are designed to increase the yield of antineoplastic agents per number of fermentations required.

The biotransformation program includes microbial and chemical modification of compounds which have a structural nucleus of interest to NCI but the drug itself is either too toxic, has some ill-desired side effect, or is not quite active enough. It is hoped that with subtle select modifications made microbially, a better compound will be obtained. Already four such compounds have been obtained this year and testing is being conducted to see if they will be candidates for toxicology. An analog program as described above along with chemical modifications produces new compounds which may act by the same mechanisms of action as the parent compound, but may give some beneficial factor such as less toxicity, more activity, or better distribution.

Co-metabolism is used to obtain completely new compounds and is a fermentation technique which allows one to short circuit the normal microbial metabolism causing unusual compounds to be formed. This work has just been initiated and sufficient data is not yet available to analyze the value of this approach.

Only fermentation broths which show in vivo activity are chemically fractionated and the active compounds isolated. Biological test systems are used to monitor the compound isolation; thus by the time the final product is isolated, purified and prepared for in vivo testing, as many as 500 tests containing this material have been evaluated. The amount of prior testing allows for greater insight into the handling and testing of the compound and also a greater assuredness of the reproducibility of the material as a true active.

TABLE 2

FERMENTATION CONTRACTORS

CONTRACTORS	INVESTIGATORS	CONTRACT NO.
Bristol Laboratories	Bradner	N01-CM-77147
Bristol Laboratories	Claridge	N01-CM-43759
Georgetown University	Katz	N01-CM-43732
W. R. Grace & Co.	Calton	N01-CM-67074
Litton Bionetics (Project 17)	Langlykke	C02-5423
Michigan Department of Health	Schuurmans	N01-CM-33702
Microbial Chemistry Research Foundation	Umezawa	N01-CM-57009
Parke-Davis & Co.	Dion	CM7-7146
Rutgers University	Nickerson	N01-CM-67106
Upjohn Company	Neil	N01-CM-77100

Plant and Animal Section

The operation of the plant products area is divided into three main areas; procurement, preparation of extracts for screening, and isolation studies of active extracts.

The initial procurement of plants comes from two major sources, an interagency agreement with USDA and a contract with the University of Hawaii. Other plant samples are procured by purchase and by voluntary submission from interested persons worldwide. Plant submissions are screened to eliminate excessive duplication of species already tested. The preparation of plant extracts for screening is performed under a service contract by Wisconsin Alumni Research Foundation.

Worldwide Surveillance

An extensive liaisonis maintained between the Branch and the pharmaceutical and chemical industries, grantees, academic institutions, research organizations, and other governmental laboratories to maintain our knowledge of natural products research and development throughout the world. Also this liason allows us to obtain prime compounds soon after discovery for evaluation in the NCI program.

This year 256 new compounds were obtained because of this liaison,many which are active and are being obtained in larger amounts for evaluation. Close contact is maintained with the suppliers in order to provide interchange of chemical and biological data which is essential to insure efficient use of material.

A two week International visit to the Far East made direct contact with the following organizations:

Japanese Foundatin for Cancer Research
Institute of Microbial Chemistry
Shionogi and Company, Ltd.
Fujisawa Pharmaceutical Company
Takeda Chemical Industries. Ltd.
Dainippon Pharmaceutical Co., Ltd.
Kanegafuchi Chemical Industry Co.
Kitasato Institute
Sankyo Company Limited
Bristol-Banyu Research Institute., Ltd.
Meiji Seika Kaisha, Ltd.
Yamanouchi Pharmaceutical Co.
Kyowa Hakko Kogyo Co., Ltd.
Tohoku University
Kaken Chemical Co., Ltd.
Taiho Pharmaceutical Co.
Sanraku-Ocean Co., Ltd.
Kureha Chemical Co.
Toyo Jozo Co., Ltd.
Nippon Kayaku Co.
The Green Cross Corporation
Sumitomo Chemical Co., Ltd.
Kumamoto University
Ajinomoto
Tanable Seiyaku Co., Ltd.
Asahi Kasei
Daiichi Seiyaku Co.

Many natural products are obtained from all parts of the world and from all types of organizations. The following criteria are used to determine if NCI is interested in testing such materials or in requesting them.

1. Compound has some anticancer activity vs any tumor whether in NCI's system or not.
2. A novel structure never evaluated in our program.

3. Activity vs yeast, fungi, or certain bacteria.
4. Reported antiviral activity (human, animal, or bacterial.)
5. Reported enzyme inhibition of proteases, lipases, CAmp phosphodiesterase, etc.

Accomplishments

The Natural Products Branch provided many new compounds to the program (Table 4). These materials were of fermentation, plant, and animal origin. Fifty-eight of these materials have demonstrated confirmed activity in one or more of the primary screens (Step III). Five compounds have passed Decision Network II and are candidates for preclinical toxicological evaluation.

Of the 396 crystalline natural products added to our program, 140 (38%) were obtained from contracts and the remainder were obtained through worldwide surveillance.

The Branch manages a total of 21 contracts with industrial, academic, and research institutions, both domestic and foreign, and one contract at FCRC.

In summary, the following was achieved in the last 15 months by the Natural Products Branch:

1. A total of 13,124 new substances were obtained for screening.
    a. 2,518 fermentation products
    b. 1,537 animal products
    c. 8,673 plant products
    d. 396 crystalline materials
2. A total of 140 compounds were isolated and purified under contract.
    a. 65 fermentation products
    b. 52 plant products
    c. 23 animal products
3. A total of 256 compound were obtained from worldwide surveillance.
    a. 152 fermentation products
    b. 88 plant products
    c. 16 animal products
4. Five new substances were assigned to Decision Network IIA (Table
    a. 4 fermentation products
    b. 1 plant product

Approximately 3-5% of the extracts tested show reproducible activity in one of the two screens used (P-388 and KB) and from these a selection is made of which plants should be collected in large quantities for isolation studies. This selection is based on the level of activity of the confirmed extracts and phytochemical knowledge of the constituents of the genera and species showing activity.

Those plants selected for isolation studies are collected in 300-500 pound quantities and sent to one of four contract laboratories for fractionation work. The progress of fractionation of the plant extracts is monitored by testing fractions in the PS or KB system and pursuing isolation studies only on the active portion of the fractionated materials. Fractionation is continued until the active materials are isolated in pure form. The purified materials are tested thoroughly and screening data is closely monitored by Natural Products Branch staff. Structural studies are performed on active compounds by the fractionation contractors. The production of active novel compounds from this approach has been satisfactory.

The animal products area is handled in similar fashion except that the isolation contractors are also responsible for initial collection and preparation of extracts of new organisms as well as fractionation. The main emphasis in this area is on marine organisms which is a relatively new area and should lead to discovery of novel active compounds unrelated to those from the plant or microbial areas. Initial compounds indicate that the marine area will indeed yield completely different compounds than those found in the terrestrial world.

When compounds isolated from the above studies are required in large quantities the material is procured and sent to a pilot plant contractor for extraction and compound isolation.

TABLE 3

PLANT AND ANIMAL PRODUCTS CONTRACTORS

CONTRACTORS	INVESTIGATORS	CONTRACT NO.
Agriculture, U.S. Dept. of	Perdue	Y01-CM-40001
Arizona State University	Pettit	N01-CM-67048
Hawaii, University of	Sagawa	N01-CM-33747
Illinois, University of	Farnsworth	N01-CM-67090
Monsanto Research Corporation	Beemsterboer	N01-CM-77140
Oklahoma, University of	Weinheimer	N01-CM-67108
Polysciences, Inc.	Boettner	N01-CM-77070
Purdue University	Cassady	N01-CM-67091
Research Triangle Institute	Wall	N01-CM-67089
Virginia, University of	Sneden	N01-CM-67002
WARF Institute	Dennis	N01-CM-43707

5. Twenty-one contracts were supervised by the Branch (Tables 2 & 3).
    a. 10 fermentation
    b. 7 plant
    c. 2 animal
    d. 2 plant prep laboratories

The major accomplishments were:

1. Success in isolation of unique organisms.
2. Cost effectiveness of pre-screening fermentation broths.
3. High number of novel compounds isolated from natural products.
4. Scale-up production of interferon (300 gal tanks).
5. Production of carboxypeptidase G.
6. Initiation of new plant extraction process which increased the number of actives in the program.
7. Large collections of marine organisms which will allow the isolation of enough of the active compounds for thorough evaluation as antineoplastic agents.
8. Effectiveness of overseas liaison with Japanese concerns. Worldwide Japan is the "hot bed" of natural products research.
9. Collection of enough Maytenus plant to isolate sufficient Maytansine for phase II clinical trials.

TABLE 4

MATERIALS ACCESSIONED AND ACTIVITY REPORT[1]

	Extracts & Fractions[4]		No.Actives		Crystalline Material Submitted		Step III[2]		Dec. PT.II[3]		INDS filed	
	1975	1976	1975	1976	1975	1976	1975	1976	1975	1976	1975	1976
Fermentation Products	1,328	2,518	141	92	159	217	29	19	3	4	1	0
Animal Products	2,086	1,537	102	90	38	39	8	0	1	0	1	0
Plant Products	6,393	8,673	346	567	155	140	30	39	1	1	2	0

1. Based on January 1976 - April 1977

2. Showing confirmed activity

3. Selected candidates for pre-clinical toxicological evaluation and clinical trials

4. 15 month count January 1976 - April 1977

TABLE 5

COMPOUNDS PASSING DECISION NETWORK IIA

NSC#	COMPOUND NAME	SOURCE
191959	Michicarcin	Fermentation
218321	2-Deoxycoformycin	Fermentation
219877	Rachelmycin	Fermentation
153353	L-alanosine	Fermentation
259968	Bourvardin	Plant

Publications by Contractors:

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ANNUAL REPORT

PHARMACEUTICAL RESOURCES BRANCH

DEVELOPMENTAL THERAPEUTICS PROGRAM

DIVISION OF CANCER TREATMENT

NATIONAL CANCER INSTITUTE

The Pharmaceutical Resources Branch, established in July, 1976, is structured to provide comprehensive pharmaceutical services to the various Programs of the Division of Cancer Treatment. The primary objectives of the Branch are to supply high quality chemical substances and formulated products for investigative program use. These objectives are accomplished essentially through contract support activities. The major contract areas include: chemical preparation and pilot plant production; analytical services; pharmaceutical research and development; and pharmaceutical manufacturing. The synthesis and distribution of radiolabeled chemicals and drugs are also provided through PRB contract sources. Additionally, the Branch is responsible for storage, distribution and inventory maintenance of all drug products used in the clinical programs.

TABLE I

RESOURCE CONTRACTS MONITORED BY
THE PHARMACEUTICAL RESOURCES BRANCH

Chemical Preparation	9
Radiolabel Synthesis	2
Pharmaceutical R & D	1
Pharmaceutical Production	4
Analytical Services	2
Drug Distribution	1
Total Resource Contracts	19

The Branch is also responsible for direct purchase of chemicals and drug products utilizing NIH procurement services.

A formulation research laboratory on the NIH campus is operated and staffed by the Pharmaceutical Resources Branch. This laboratory is assigned research projects of high program interest which present difficult drug delivery problems. Most of the chemical agents developed by the Laboratory of Medicinal

Chemistry and Biology are assigned to this laboratory. This arrangement facilitates a scientific exchange between the developer and formulator and a team approach to the ultimate product design.

Branch Staff

The Pharmaceutical Resources Branch is presently staffed with six senior professionals, two technical and three clerical personnel. In addition, two Visiting Fellow positions are assigned to the Formulation Laboratory. The classification of the senior professionals is as follows: three PHS Commissioned Corps pharmacists; one analytical chemist; one Ph.D. medicinal chemist; and one staff fellow.

The Branch consists of two Sections and one operational unit within the Office of the Chief.

1. Chemical Resources Section - Head, Dr. Robert R. Engle

    The primary functions of the Chemical Resources Section are to provide for resynthesis, large-scale production and procurement services for acquisition of chemical substances. These services are accomplished by the management and supervision of a contract program for (a) resynthesis of small quantities of bulk substances as required for preclinical assessment and (b) pilot plant production of various quantities of bulk substances intended for pharmaceutical manufacture of clinical investigational products. The Section also initiates the acquisition of commercially available chemical substances through NIH procurement contract procedures. This requires the preparation of stringent material specifications to insure that high quality products are obtained.

    Another major function of the Section involves the management of a contract program to prepare and distribute radiolabeled materials. These materials are distributed to authorized investigators for clinical pharmacology and other related studies.

    This Section supervises nine chemical prep lab contracts and two radiolabel synthesis laboratory contracts.

2. Analytical and Product Development Section - Head, Mr. James C. Cradock

    This Section is responsible for the quality assessment of chemicals and formulated drugs used in the Division of Cancer Treatment's drug evaluation program. Analyses are conducted by contract laboratories independent of chemical or formulated drug suppliers. This Section is also responsible for the development of analytical methodology to determine the purity of chemicals, potency of active ingredients in pharmaceutical formulations, stability of formulated products under accelerated and simulated use conditions and identification of impurities and/or degradation products. Other evaluations include determination of partition coefficients for use by other DTP components in structure activity studies and application of analytical techniques to estimation of certain compounds in biological fluids. Data are used for preparation of analytical profiles of bulk drugs, designation of reference standards and preparation of purchase specifications of bulk chemicals.

A second major function regards the development of suitable pharmaceutical dosage forms for clinical trial. Since most preparations are intended for intravenous use, studies are undertaken to assess the solubility and stability in a variety of pharmaceutical vehicles. New approaches to enhance solubility are emphasized since few suitable methods are available to the formulator of parenteral products.

Most of the product development effort is conducted under contract with the Section staff serving as project monitors. In addition, an intramural formulation laboratory supervised and maintained by this Section is investigating methods to solve drug formulation problems.

This Section supervises a total of four contracts: two analytical contracts; one formulation research contract; and one small-scale production contract. An additional contractor for the development of radioimmunoassays of otherwise poorly characterized drugs will be added to the Section's responsibilities during the next fiscal year.

3. Office of the Chief, Clinical Products Unit - Mr. Larry M. Kleinman

This Unit is responsible for a number of functions involving the final dosage forms for investigational trials. The major functions include the management of three pharmaceutical production contractors; the management of clinical drug distribution and inventory contract; and the direct purchase of all products used in the various clinical programs.

This Unit prepared all product and label specifications on contractor produced investigational products. Pharmaceutical data sheets for Clinical Brochures are also prepared from this Unit.

In addition to the management of the pharmaceutical and distribution resource contracts, this Unit manages a sizeable intramural budget for the direct purchase of chemicals and formulated products. During this reporting period, the drug purchase expenditures were in excess of $5,000,000. A significant amount of staff time is expended in this area in preparing purchase specifications, award justifications and periodic budget projections throughout the year. Several types of NIH contract mechanisms are utilized for these procurement actions including bids from suppliers, blanket purchase orders, direct purchase contracts, etc.

Goals and Accomplishments

The Branch successfully accomplished its objectives during this reporting period by providing efficient chemical and pharmaceutical services to the Programs of the Division of Cancer Treatment. For example, more than 120 kg of high quality methotrexate was produced for high dose studies. This large quantity of drug could not be obtained commercially in sufficient quantity to prevent delays in the ongoing Clinical Programs. The contract preparation of this chemical and the ultimate formulation by other Branch contractors resulted in a considerable financial savings to the Division. Other chemicals of high quality produced by the chemical prep laboratories included PALA (NSC-224131), pentamethylmelamine hydrochloride (NSC-118742), the nitrosoureas, etc.

The Analytical and Product Development Section continued to provide superior analytical contract services to the program. In addition to the routine analytical evaluations of chemical and formulated products, these contractors continue to upgrade analytical methodology. As a result of these efforts, improvements in methodology were made on methotrexate, daunorubicin and other drugs of high importance to the Division. Also, a reference standards program was begun during the past year. This program was considered necessary for improved formulation activities and in conformance to good manufacturing practices of the Food and Drug Administration.

Several formulated products were developed during this reporting period that required development of non-conventional parenteral vehicle systems. For example, methanesulfon-m-anisidide, 4'-(9-acridinyl-amino-), NSC-249992 is provided in a dimethylacetamide solution which must be further diluted at the time of use with a lactic acid vehicle. This product has completed large animal toxicology and will be filed with the FDA. A parenteral form of 1-(2-chloroethyl)-3-(2,6-dioxo-3-piperidyl)-1-nitrosourea (PCNU, NSC-95466) was also developed for preclinical toxicological assessment. Other achievements of the formulation development group are described in the Section report.

The contract pharmaceutical production activity continued, as in the past, at a high level of effort. These contractors produced almost one million parenteral units and more than 1.6 million oral dosage units (tablets and capsules). The investigational product cis-diamminedichloroplatinum (II), NSC-119875 has the highest clinical interest and demand. During this reporting period, over 300,000 vials were produced.

The radiolabel syntehsis contractors successfully achieved the preparation of ^{14}C labeled adriamycin and tritium labeled vincristine. They also provided 27 additional labeled materials for research use. The requirements for radiolabeled materials is increasing and both contractors continue to perform at the maximum level of effort.

The service requirements of the Branch continue to increase. Our objective is to be prepared for all new challenges and to maintain a contract capacity to provide efficient pharmaceutical support to the program.

A preparation laboratory contractors meeting was held during this reporting period to discuss new techniques and procedures. This was a highly successful workshop and similar workshops are being planned.

The Branch actively participated in several professional meetings during this reporting period.

1. American Society of Hospital Pharmacists (ASHP)

    a. J. Paul Davignon, Chairman 1976-1977, Special Interest Group on Oncology Pharmacy Practice (SIG).

    b. Cradock, J. C., Kleinman, L. M. and Rahman, A. The Intrathecal (I.T.) Route: Evaluation of Some Pharmaceutical Factors, Poster Session, 11th Annual ASHP Midyear Clinical Meeting, Anaheim, California, December 5-9, 1976.

    c. Kleinman, L. M. and Davignon, J. P. Investigational Anticancer Agents, Poster Session, 11th Annual ASHP Midyear Clinical Meeting, Anaheim, California, December 5-9, 1976.

    d. Kleinman, L. M. SIG on Oncology Pharmacy Practice, Curbside Session, Expert on Investigational Drugs, 34th Annual Meeting of ASHP, New York, New York, May 14-19, 1977.

2. Parenteral Drug Association

    a. Cradock, J. C., Kleinman, L. M. and Davignon, J. P. Intrathecal Injections - A Review of Pharmaceutical Factors, Annual Convention of the Parenteral Drug Association, San Francisco, California, November 3-5, 1976.

3. Academy of Pharmaceutical Sciences, American Pharmaceutical Association

    a. Rahman, A., Cradock, J. C. and Davignon, J. P. Carrier Mediated Drug Delivery: Formulation Studies with an Adriamycin-DNA Complex, 21st National Meeting, Orlando, Florida, November 14-17, 1976.

    b. Rahman, A., Cradock, J. C. and Davignon, J. P. Study of the Dissolution and Absorption of the Antineoplastic Agent Ellipticine, 21st National Meeting, Orlando, Florida, November 14-17, 1976.

Publications by Contractors

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CHEMICAL RESOURCES SECTION

PHARMACEUTICAL RESOURCES BRANCH

Scope

The function of the Chemical Resources Section is to procure bulk chemicals and drugs for confirmational and other testing, toxicological and pharmacological evaluation, and clinical trials in humans; and to procure radiolabeled materials for pharmacology, clinical pharmacology and related studies. The function of the Section is achieved through the management and supervision of a contract program for the preparation of various quantities of bulk chemicals and drugs and radiolabeled materials, and the acquisition of commercially available chemical substances.

Staff

The staff of the Chemical Resources Section consists of one professional person and a secretary. The professional staff member serves as the project officer on the various contract activities of the Section.

Preparative Activities

The resynthesis or preparation laboratories are, in the strictest sense, service laboratories, and are designed and selected to prepare known chemicals and bulk drugs which are needed by the Program. The compounds selected for preparation are not readily available in the quantities needed from the original supplier or on the open market.

These laboratories are also used to obtain data for the preparation of the necessary quantities of clinically-important chemicals and to develop the most economical means for their preparation. It should be pointed out that many methods of synthesis which are practical for small quantity are not technically feasible or economically practical when used for a large scale synthetic operation or for radiolabel synthesis. The conversion of small-scale to large-scale production often requires developmental studies which are conveniently carried out by the preparation laboratories. Solubility and stability studies, and cost data are also provided by these contractors. These contractors have, on occasion, provided materials in formulated dose form.

The increased emphasis in clinical investigations has caused a change in the preparation activities. The increased effort being devoted to toxicological and clinical studies has resulted in the need for larger quantities of the compounds prepared by this program. Also, the decision to evaluate a large number of compounds demonstrating confirmed activity in the initial screening in the tumor panel has also resulted in the need for larger quantities of the compounds being resynthesized for preclinical evaluation. This change in emphasis has been handled by the preparation laboratories without difficulty.

The preparation laboratories, taken collectively, provide the means of obtaining nearly any type of chemical compound, regardless of structure, and the ability of providing large quantities of very high purity drugs.

1. Bulk Chemicals and Drugs

The compounds chosen for resynthesis at the preparation laboratories are assigned for resynthesis for five major reasons: (a) clinical investigations; (b) preclinical toxicological and pharmacological evaluation; (c) maintaining a stock of chemicals of clinical and preclinical interest; (d) completion of screening or confirmational testing; and (e) use as intermediates in further synthesis. The compounds so chosen are placed on priority lists based upon their relative importance, with clinical compounds given top rating. The quantity of a given material to be resynthesized may vary from 1 gram to 200 kilograms. Factors governing the amounts depend upon: use, ease of preparation, stability, and cost. Materials of high quality are prepared in the preparation laboratories. Every effort is made to safeguard the patient in the quality of the drugs used.

The Chemical Resources Section, through the preparative laboratories, directed the resynthesis of 239 compounds totaling 350 kilograms during this report period. Included in this group were 62 compounds scheduled for or undergoing clinical evaluation totaling 303 kilograms. In addition to the compounds resynthesized, 138 new compounds were also prepared by the preparative laboratories. The Section also purchased 14 compounds scheduled for or undergoing clinical evaluation totaling 146 kilograms. In addition, the Section obtained as gifts from various organizations a total of 11 compounds totaling 61.5 kilograms which were scheduled for toxicological or clinical evaluation.

During this report period large quantities (more than 120 kilograms) of Methotrexate (NSC-740) were prepared by a preparation laboratory at a substantial saving (less than one half) over the previous purchase price. This material was prepared via the new process devised by Southern Research Institute (Contract N01-CM-43762) and developed by the preparation laboratories. The quality of the Methotrexate prepared was very high. In addition, a good procedure for the preparation of kilogram quantities of dichloromethotrexate and aminopterin has been developed and the preparation of the kilogram quantities of dichloromethotrexate needed for clinical studies are being prepared. A method of synthesis suitable for the preparation of the large quantities of PALA (NSC-224131) needed for pre-clinical and clinical studies has been developed and the preparation of kilogram quantities is in progress. Also, the preparation laboratories responded rapidly to the preparation of the large amounts of pentamethylmelamine hydrochloride (NSC-118742) and other hexamethylmelamine derivatives requested by the program.

The Section directed nine contract laboratories for the preparation of materials needed by the Program (Table II).

TABLE II

CONTRACT LABORATORIES FOR PREPARATION OF CLINICAL AND EXPERIMENTAL COMPOUNDS

Contractor	Investigator	Contract No.
Aldrich Chemical Company	Wickersham/Szabo	N01-CM-23706
Ash Stevens, Inc.	Ash	N01-CM-57012
Cordova Chemical Company	Olsen	N01-CM-57031
Dow Chemical Company	Collins/Bublitz	N01-CM-53768
Monsanto Research Corporation	Ellard	N01-CM-67046TQ
Parke, Davis & Company	Short/McMillan	N01-CM-23708
Pharm-Eco Laboratories, Inc.	Draper	N01-CM-23707
Starks Associates, Inc.	Starks	N01-CM-23203
Starks Associates, Inc.	Schultz	N01-CM-53769

2. Radiolabeled Materials

The Section coordinates the procurement and distribution of radiolabeled chemicals and drugs for pharmacological and drug distribution studies. Materials not available from commercial sources are prepared by contract preparative laboratories. These contracts also handle the storage and distribution and perform the necessary analytical work for all labeled materials, whether prepared under the contracts or acquired from other sources. All requests for radio-active materials are reviewed by a committee in relation to proposed use prior to assignment to the contracts for procurement. During this report period the preparation of ^{14}C labeled Adriamycin (NSC-123127) was achieved and tritium labeled Vincristine (NSC-67574) was obtained. A total of 27 radiolabeled materials (Table III) have been procured from all sources. Two hundred and twenty-two shipments of radio-active substances were made during this report period.

TABLE III

RADIOLABELED MATERIALS PROCURED

NSC No.	Radiolabeled Materials	Label
740	Methotrexate	^{3}H (3',5')
1390	Allopurinol	^{14}C (2)

NSC No.	Radiolabeled Materials	Label
3088	Chlorambucil	^{14}C (Ring)
3088	Chlorambucil	^{14}C (Ethylene)
8806	Melphalan	^{14}C (Ethylene)
19893	Uracil, 5-fluoro-	^{14}C (2)
49842	Vinblastine	^{3}H (General)
51143	1H-Imidazo(1,2b)pyrazole, 2,3-dihydro	^{14}C (2,3)
67574	Vincristine	^{3}H (General)
73754	Fluorodopan	^{14}C (Chloro-ethyl-2)
79037	Urea, 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea (CCNU)	^{14}C (Ethylene-2)
82151	Daunorubicin	^{14}C (14)
102816	5-Azacytidine	^{14}C (4)
104801	Acrylic acid, 3-(*p*-anisoyl)-3-bromo-sodium salt (Sodium Bromebrate)	^{14}C (Methyl)
112907	Tetrahydrouridine	^{14}C (2)
123127	Adriamycin	^{14}C (14)
126849	3-Deazauridine	^{14}C (6)
139490	Tetrahydrohomofolic acid, 5-methyl-, sodium salt	^{14}C (Methyl)
141549	Methanesulfon-*m*-anisidide, 4'-(9-acridinylamino)-, monohydrochloride	^{14}C (Anisidide)
148958	Ftorafur	^{14}C (2)
172112	1,3-Diazaspiro[4,5]decane-2,4-dione, 3-[2-[bis(2-chloroethyl)amino]ethyl]- (Spirohydantoin Mustard)	^{14}C (Hydantoin-4)

NSC No.	Radiolabeled Material	Label
172112	1,3-Diazaspiro[4,5]decane-2,4-dione, 3-[2-[bis(2-chloroethyl)amino]ethyl]-(Spirohydantoin Mustard)	^{14}C (Chloroethyl)
176319	Quinolinium, 6-amino-1-methyl-4-[[[[[[(1-methylpyridinium-4-yl)amino]phenyl]amino]-carbonyl]phenyl]amino]-, dibromide	^{14}C (Quinoline)
218321	Co-vidarabine	^{3}H (General)
264074	1,2-Ethylene-3,3'-bis(5,5-pentamethyl-enehydantoin)	^{14}C (Hydantoin-4)
404241	Adenine, 9-beta-*D*-arabinofuranosyl-(*Ara*-A)	^{3}H (2)

The Section directed three contracts devoted to the procurement of radiolabeled materials (Table IV).

TABLE IV

CONTRACT LABORATORIES FOR

PREPARATION OF RADIOLABELED MATERIALS

Contractor	Investigator	Contract No.
Monsanto Research Corporation	Yanko	N01-CM-23715*
Research Triangle Institute	Kepler	N01-CM-67121
Stanford Research Institute	Leaffer	N01-CM-63776

*Terminated 12/31/76

Contractors Workshop

During the past year a Preparation Laboratory Contractors Workshop was held with representatives from all the Preparation Laboratory Contractors in attendance. The major objective of this meeting was for the different contractors in this area of endeavor to discuss mutual problems, techniques and procedures. In addition, the Section Staff had an opportunity to review administrative procedures and to inform the contractors of changes in the

Program. Invited speakers made presentations of interest to the group and the contractors discussed topics of mutual interest. A favorable response to this workshop was expressed by all in attendance and the contractors requested that additional workshops of this type be held. Additional workshops of the contractors in a specific area are being planned.

ANALYTICAL AND PRODUCT DEVELOPMENT SECTION

PHARMACEUTICAL RESOURCES BRANCH

About one year ago the analytical chemistry and dosage form development activities were merged into one section within the Pharmaceutical Resources Branch. Major responsibilities of the Section fall into two distinct categories:

I. Analytical Chemistry

a. Development of suitable methodology for the analysis and characterization bulk chemicals, and pharmaceutical dosage forms.

b. Development of specifications for use in procurement of bulk chemical agents.

c. Serve as a repository for all methods of preparation and analytical characterization of bulk chemicals. The data are summarized in a Chemical Information Sheet and made available to investigators using this material.

II. Dosage Form Development

a. Characterization of the pharmaceutical properties of new chemical agents, development of dosage forms, and evaluation of chemical and physical changes under simulated use conditions.

b. Development of new approaches for enhancing solubility and stability of selected antitumor agents.

This Section is staffed by one pharmacist, one analytical chemist, one staff fellow, two visiting fellows, and one secretary.

A. Analytical Chemistry

Clinical and preclinical evaluations of antitumor agents require availability of bulk chemicals and pharmaceutical dosage forms of highest quality. These materials must be subjected to rigorous assessment of their identity, purity and quantitative composition by definitive analytical methods. Analyses are conducted by contract laboratories independent of chemical or formulated drug suppliers. The analytical data are utilized for a number of chemical and pharmaceutical applications within the Pharmaceutical Resources Branch including:

1) Preformulation assessment of solubility and stability as well as analytical characterizations that are of benefit in the design of dosage form development studies.

2) Establishment of procurement specifications for bulk chemicals acquired on a bid basis.

3) Assembly of analytical data on bulk drug substances and pharmaceutical dosage forms for submission to the Food and Drug Administration. Analytical data are retained on all bulk materials used in clinical trial under the sponsorship of the Division of Cancer Treatment.

In addition, information acquired from these analytical laboratories is condensed into a "Chemical Information Sheet." This compilation is a part of the Clinical Brochure which is distributed to the various investigators. The data provide information on the characterization of the drug substance, serves as a guide for safe handling during storage and shipping, and provides preliminary chemical and solubility data potentially applicable to extraction and estimation of the drug in biologic samples. In addition, partition coefficients are determined in octanol-water systems for applition to structure activity studies.

The determination of the purity of a new chemical entity is often an analytcal research problem requiring the use of a combination of sophisticated techniques as well as consideration of the stability of the compound and its possible contaminants or decomposition products. Among the techniques that are most commonly used in drug analysis are: spectroscopic (ultraviolet, visible, infrared and nuclear magnetic resonance), chromatographic (paper, thin layer, high pressure liquid, and gas-liquid) and titrimetric (aqueous and potentiometric). In addition to the above, elemental microanalysis is carried out. Where applicable, optical rotation, optical rotatory dispersions, refractive index, functional group assay, the degree of hydration or solvolysis determinations and the preparation of derivatives are performed. Chromatographic procedures are most useful in the determination of the presence of a possible unidentified impurity. The combination of this procedure with the melting point, infrared and ultraviolet spectra is often sufficient for the analysis of a simple, stable, well-characterized compound.

The analytical contractors are continually reevaluating and upgrading methodology. Improved high presure liquid chromatography methods were developed for a number of agents including methotrexate and daunorubicin. Both of these drugs are administered clinically at very high dosage. Quantitative detection of impurities is essential. A thorough evaluation of the purity and stability of 3-deazauridine was also conducted. The data demonstrated that this agent is stable at room temperature for extended periods.

A reference standards program was begun during the past year. One bulk lot is designated as the reference and made available primarily to contractors within the Pharmaceutical Resources Branch. This batch is of high quality and is selected on the basis of chemical purity from two or three lots of comparable size.

During fiscal year 1976 and the transition quarter, there were 248 analytical reports on 79 different compounds. These reports consisted of the analysis of 141 lots of bulk chemicals of clinical interest (704.5 kg), six lots of bulk materials, 44 lots of clinical formulations for release to clinical investigators, and 38 lots of clinical formulations for periodic shelf life evaluation.

Assistance was also extended to the formulation facilities and the preparation laboratories with their analytical and formulation problems concerning compounds of interest to the program.

The Section directed two analytical-quality control contract laboratories (Table V). In addition to the two analytical laboratories, elemental analyses are obtained from Micro-Analysis, Inc., under a purchase order agreement.

TABLE V

CONTRACT LABORATORIES FOR ANALYTICAL-QUALITY CONTROL

Contractor	Investigator	Contract No.
Midwest Research Institute	Haggerty	N01-CM-33722
Stanford Research Institute	Lim	N01-CM-33723

A problem of increasing magnitude involves the characterization and analysis of certain natural products, particularly proteins and glycoproteins. Acquisition of a contract resource in this area is anticipated within the coming year.

B. Dosage Form Development

Formulation development commences when an agent passes Decision Network IIA and sometimes continues into the clinical trial. The first approach to development involves an evaluation of existing physical and chemical data as well as in vivo route and regimen studies in rodents. These results serve to define the scope of the formulation problem. Relatively straightforward development projects are assigned directly to the contract laboratory equipped with production scale capabilities. The contractor is provided with available chemical data, estimated dose and intended route of administration. Consultation with the Project Officer is carried out regarding formulation approaches. The chemical is subjected to solubility and stability studies in a selected group of physiologically acceptable vehicles. An analytical method is usually devised for detection of the parent compound in the presence of excipients and/or degradation products. The influence of pH, temperature, light, oxygen, packaging components and pharmaceutical additives is assessed on the stability of drug solutions. Pilot batches are then prepared under conditions simulating production scale. Physical and chemical stability of the dosage form is assessed under accelerated use conditions.

Some of the drugs recommended for development as intravenous dosage forms are poorly water soluble. Since biologically suitable solubilization techniques are limited, low solubility has been a problem in preparation of parenteral dosage forms. Newer approaches have been investigated under a contract with the University of Kansas and in the Pharmaceutical Resources Branch Formulation Laboratory. A series of bis-3-carboxy-4-hydroxy-1-naphtyl-alkanones were evaluated as complexing agents at the University of Kansas. The stability of selenoguanosine and beta thioguanosine was evaluated in alkaline media. The seleno derivative readily decomposed, but approach has been devised to yield a stable infusion solution.

Research efforts in the formulation laboratory have been described in the project report but will be briefly summarized. A series of substituted benzoic acid derivatives markedly increased the solubility of chartreusin and experimental pilot scale formulations were prepared. The Adriamycin-DNA complex was evaluated as an approach to selective drug delivery. Good agreement was demonstrated between viscometric determinations and pharmacokinetic behavior. A product has been successfully manufactured on a small production scale. Thermal denaturation during the sterilization (autoclaving) process was the major problem. Sodium chloride 0.1-0.15 M retarded inactivation but careful monitoring of autoclave temperature was essential. Unique capabilities in development and production have permitted the University of Iowa to play an increasing important role in our program. A major accomplishment during the past year involved a study of the stability of thymidine and the subsequent development of a stable, high dosage infusion solution. Previously, a more expensive and unwieldy lyophilization process was used.

Experimental formulations are submitted for evaluation and confirmation of antitumor activity. Simultaneously, with these studies, the dosage forms are sent to an independent laboratory for chemical and/or biological analyses. The toxicological results and the accumulated pharmaceutical development data serve as a basis for determining the starting human dose and the ultimate dosage form to be used in the initial clinical studies Several dosage strengths of the same investigational drug may be prepared provided the drug concentration remains within the solubility limits of the active ingredient.

During 1976, 33 projects for dosage form development were assigned. Twelve of these involved new drug research and twenty-one assignments were for modification of existing preparations.

TABLE VI

CONTRACTS FOR DOSAGE FORM DEVELOPMENT

Contractor	Investigator	Contractor No.
Iowa, University of	Wurster	NO1-CM-43743
Kansas, University of	Higuchi	NO1-CM-23217

CLINICAL PRODUCTS UNIT

PHARMACEUTICAL RESOURCES BRANCH

Several functions performed by the Pharmaceutical Resources Branch come under the Office of the Chief. The staff responsible for these functions comprises the Clinical Products Unit. These functions are as follows:

1. Production and packaging of investigational drugs;
2. Procurement by direct purchase of formulations for clinical use;
3. Quality control surveillance of all clinical dosage forms;
4. Preparation of pharmaceutical data sheets for Clinical Brochures, IND's, etc.;
5. Storage and distribution of clinical dosage forms.

Production and Packaging of Investigational Drugs

The Branch has the responsibility to provide the clinical programs with adequate quantities of formulated materials. The production of investigational dosage forms is mainly performed by contract agreement with qualified pharmaceutical manufacturers. These contractors provide the Branch with a capability of producing a broad range of pharmaceutical products. The production output of clinical dosage forms for the period covered by this report remained at a high level of effort. The formulation contractors produced a combined total of 987,124 vials (parenteral doses) and 44,009 bottles of tablets and capsules (1,633,870 oral dosage forms). Production output which is dictated by clinical needs has continued to increase during the last few years. The production contractors have been able to meet the program's needs for a variety of formulated products. Four contracts were monitored by the Clinical Products Unit.

TABLE VII

CONTRACTS MONITORED BY THE CLINICAL PRODUCTS UNIT

Contractor	Investigator	Contract No.
Ben Venue Laboratories, Inc.	Wickes	N01-CM-67109
Philips Roxane Laboratories, Inc.	Marine	N01-CM-67053
Elkins-Sinn, Inc.	Reese	N01-CM-67103

Contractor	Investigator	Contract No.
Flow Laboratories, Inc.*	Dembeck	N01-CM-67088

*This contractor is responsible for clinical drug storage and distribution.

Some investigational products are obtained from non-contract pharmaceutical sources on a contributed or cost sharing basis. These products are usually provided in exchange for access to clinical data generated by the investigative trials with the product. The dosage forms are prepared from specifications which are established by mutual agreement between the Branch and the manufacturer. During the reporting period, the following companies supplied a total of 129,775 injectables and 769 bottles of tablets and/or capsules through this joint research effort:

Bristol Laboratories, USA	NSC-157365	Neocarzinostatin
Eli Lilly and Company, USA	NSC-143095	Pyrazofurin
Sandoz, USA	NSC-122819	VM-26
Sandoz, USA	NSC-141540	VP16-213
Wellcome Foundation, England	NSC-220537	Corynebacterium Parvum
Winthrop Laboratories, USA	NSC-142982	Hycanthone
Janssen Pharmaceuticals, USA	NSC-177023	Levamisole
Richardson-Merrell, USA	NSC-151466	Enclomiphene
Rhodia, Inc., France	NSC-164011	Rubidazone

Purchase of Dosage Forms

Another activity of the Unit involves the direct purchase of formulated products. These items fall into two product categories: (1) investigational products; and (2) commercial products. Investigational products are usually manufactured according to NCI specifications and sold to NCI on a cost reimbursement basis. Commercial products are items available for open purchase. Procurement of all direct purchased dosage forms is handled through the NIH Procurement Branch. Several procurement mechanisms are used depending on such factors as the type of product, the quantity required and the priority for clinical need.

A total of 636,112 vials and 225,088 bottles of tablets and/or capsules (5,068,420 oral doses) of direct purchased items at a cost of $4,653,925.00 were obtained during the 15 months of this reporting period. All suppliers are required to provide a certificate of analysis on each lot of drug purchased. The data are reviewed prior to release for clinical use.

The table below summarizes the procurement activities of the Clinical Products Unit, Office of the Chief.

TABLE VIII

DRUG ITEMS PROCURED

	Capsules/Tablets	Injectables
Produced by Contractors	1,663,870	987,124
Contributed or Cost Sharing	278,600	129,775
Purchased Investigational Drugs	182,295	216,389
Purchased Commercial Drugs	2,943,655	419,723
	5,068,420	1,753,011

Quality Control of Clinical Dosage Forms

Each lot of formulated drug must pass specific quality control criteria prior to release for clinical use. The Clinical Products Unit in collaboration with the two Sections in the Branch, maintain a strict quality control program designed to insure that each batch of formulated material meets the criteria for strength, quality and purity. Parenteral products are tested for potency, sterility, safety, pyrogenicity, etc., while oral products are tested for content uniformity, weight variation, hardness, disintegration, dissolution, etc. All products are tested for potency by the formulator with random testing by an analytical contractor. All new dosage forms are routinely submitted to an analytical contractor to verify the analytical methodology and accuracy of the formulator.

The pharmaceutical contractors are requested to perform compatibility and stability studies on reconstituted and diluted solutions of parenteral investigational dosage forms. These studies are carried out under the simulated conditions of the clinical setting. The accumulated information is then reported on a Pharmaceutical Data Sheet which is incorporated into the Clinical Drug Brochure.

The Clinical Products Unit, Office of the Chief, maintains a shelf life surveillance program on all lots of contractor produced investigational dosage forms. Representative samples of each lot are stored under specified controlled conditions and assayed for chemical and/or physical change. Three assay schedules are used for shelf life studies: Schedule A, designed to provide stability data under accelerated conditions; Schedule B, designed for studies of repeat formulations; and Schedule C, assigned to products that have been prepared more than three times. This last schedule involves the retention of samples for assays to be performed on request. All schedules provide a minimum of four years surveillance.

Preparation of Pharmaceutical Data Sheets

The Clinical Products Unit is responsible for the preparation of Pharmaceutical Data Sheets for all investigational dosage forms used in the program. The data sheets are used in the preparation of Clinical Brochures and are disseminated to health care practitioners. These sheets provide reconstitution, stability and precautions to be used in the handling of the dosage form.

The Clinical Products Unit also prepares Investigational New Drug (IND) Attachments I, II, III, V and VII for submission to the Investigational Drug Branch for FDA-IND filing. These attachments consist of manufacture, control, packaging and labelling data required for the investigational dosage forms. The Pharmaceutical Resources Branch submitted attachments for four (4) original IND's and sixteen (16) amended IND's to the Drug Liaison and Distribution Section, IDB.

Storage and Distribution of Clinical Dosage Forms

In 1976, the storage and distribution of clinical drugs was contracted to Flow Laboratories, Inc. This was the direct result of an ever increasing demand for clinical products supplied by the Division. The transfer of this activity was carried out smoothly and without interruption in service to the clinical investigators. During the reporting period, a total of 1,878,009 drug items (vials and bottles) were received and inventoried for subsequent worldwide distribution. During the same period, 1,880,837 drug items were distributed averaging 51 individual shipments daily.

During this report period, an average of 123 different formulations were maintained on the inventory. These items include investigational and commercial products.

Significant Accomplishments

The pharmaceutical production contractors of the Branch were able to meet an increased clinical demand for several high interest drugs. The production contractors produced 304,924 vials of cis-diamminedichloroplatinum (II); 162,502 vials of methotrexate (all strengths); 98,598 vials of daunorubicin; 79,876 vials of ftorafur; and 52,494 vials of calcium leucovorin.

The Unit worked with a production contractor to modify his existing manufacturing procedures and equipment to allow for increasing the batch size of 5-azacytidine vials. This unstable product can now be produced in 12,000 vial batches instead of the previous 5,000 vial batch. This drug has an increasing rate of distribution which necessitated modification for larger batches.

The storage and distribution functions previously performed by in-house personnel of the Unit were contracted to Flow Laboratories, Inc. The transistion was affected without an interruption in drug shipments to clinical investigators. This contractor is expected to incorporate a computerized inventory system in the coming year.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 03584-05 PRB

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Research in the Dosage Form Development of New Antitumor Drugs

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. C. Cradock	Head, Anal. & Product Dev. Sect.	PRB NCI
OTHER:	J. P. Davignon	Chief, Phar. Res. Br.	PRB NCI
	A. Rahman	Visiting Fellow	PRB NCI
	G. Poochikian	Visiting Fellow	PRB NCI
	Karl P. Flora	Staff Fellow	PRB NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH
Pharmaceutical Resources Branch

SECTION
Analytical and Product Development Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS: 2.0	PROFESSIONAL: 2.0	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project describes the intramural formulation activities with potential antineoplastic agents. The effect of formulation techniques on the physicochemical properties and bioavailability of an adriamycin-DNA complex. Intrinsic viscosity determinations indicated that the quality of the complex was influenced by duration of autoclaving and electrolyte concentration. Optimal conditions included a 15 minute autoclave cycle and an ionic strength of 0.1 M. The utility of intrinsic viscosity as a predictor of biologic response was evaluated in mice and rats. Relative to free drug, the complex produced more prolonged blood levels, a two fold increase in urinary excretion, decreased levels in mouse heart and a two or four fold increase of drug equivalents in liver. Only minor increases of drug equivalents in plasma and liver followed administration of the complex in a salt free solution. A series of substituted benzoic acid derivatives were evaluated as complexing agents using phase solubility techniques. The solubility of the antitumor agent chartreusin was related to degree and site of hydroxy substitution.

General Objectives:

1. Study new formulation approaches with potential utility for those compounds posing difficult formulation problems.

2. Evaluate dosage form problems presented by clinical and laboratory investigators.

Specific Objectives:

1. Evaluation of processing variables on the efficacy of a DNA-adriamycin complex.

2. Evaluation of substituted benzoic acids as complexing agents.

3. Evaluation of pharmaceutical additives in the low temperature vacuum drying method.

4. Assessment of the pharmaceutical properties of intrathecally administered drugs.

Major Findings:

1. DNA-Adriamycin Complexes

Complexes of antitumor agents with macromolecules have been utilized in attempts to beneficially influence antineoplastic activity or toxicity. DNA complexes of daunorubicin and adriamycin have been among the most extensively studied. Atassi and associates (Europ. J. Cancer, 11:309, 1975) reported that the method of preparation affected the biologic behavior of the complex. The multistep formulation procedure was monitored by viscometric techniques using suspended level instruments. Intrinsic viscosity was affected by autoclave time, rate of cooling, electrolyte concentration, DNA type and addition of adriamycin. The most critical factors were the ionic strength and duration of autoclaving of the DNA solution. Optimal conditions included a 15 minute autoclave cycle, slow cooling and a sodium chloride concentration of 0.1-0.15 M.

A physiologic disposition study of several formulations was conducted in rodents to assess the predictability of the viscosity determinations. In contrast to the free drug or complex in water, serum levels of adriamycin fluorescent equivalents were markedly elevated for up to two hours when administered as complex in 0.1 or 0.15 M NaCl. These latter preparations also produced higher (2-3 fold) levels of drug equivalents in liver through eight hours, but no appreciable differences between formulations were evident at 12 or 24 hours. Complex in 0.1 M NaCl produced a two fold higher level in spleen through the first four hours. Similar behavior has been noted with liposomal drug delivery. Drug equivalents in mouse heart or kidney appeared not influenced by the method of preparation, but the urinary excretion of adriamycin in the mouse was affected by complexation. About 6.8% and 13% of the injected dose was recovered in urine 72 hours after administration of free drug and complex in 0.1 M NaCl, respectively. Since the anthracyclines are principally excreted via the feces, the biliary excretion was evaluated. For the first two hours, the drug concentration in bile was 2-4 fold higher in rats administered the free drug, but no differences were evident from two to six hours after injection. The cumulative per cent of injected dose excreted was 19.6% for free drug group and 18% for the complex prepared in 0.1 M NaCl.

The formulation procedure affected the antitumor response was evaluated in the i.p. L_{1210} system. At the same dose level, the increase in life span of mice treated with DNA complexes in 0.05-0.15 M NaCl was at least double that observed with the free drug. These data indicate that the method of complexation acutely influences the disposition of adriamycin and suggests that intrinsic viscosity determinations are appropriate in vitro indicators of bioavailability of the adriamycin-DNA complex. However, in nearly all instances there were no appreciable differences between free drug and complex eight hours after injection.

2. Complexing Agents

Approaches to improving solubility are rather limited and more biologically compatible complexing agents are needed. An observation that sodium gentisate (2,5 dihydroxybenzoate) increases the solubility of the antitumor agent Chartreusin was followed by a systematic evaluation of other substituted benzoates using phase solubility techniques at 25°C. The water solubility of chartreusin (∿ 15 mg/L) was increased about 5 fold by 0.52 M sodium benzoate and solubility was directly related to concentration. However, the plots of solubility versus concentration of other ligands were sigmoidal and indicative of higher order complexes as well as 1:1 complexes. Monohydroxy derivatives substantially improved the solubility with a 60 fold increase induced by the ortho substituted compound. Dihydroxy analogues increased the solubility of chartreusin (300-400 fold). In addition, equilibrium solubility was attained more rapidly than noted with monohydroxy derivative or benzoic acid. Trihydroxybenzoic acid further increased the chartreusin solubility by about 5 fold.

Complexation is assumed to be responsible for the increase in solubility. However, surface tension studies of chartreusin-gentisate mixtures at a fixed concentration of sodium gentisate (0.26 M) and variable chartreusin concentration ($0-1.7 \times 10^{-5}$ M) showed a drop in surface tension with increasing concentrations of chartreusin to a constant minimum value. These observations suggest micelle formation is playing a role in the solubility increase.

These experimental observations were successfully applied to the preparation of lyophilized formulations of chartreusin-gentisate and chartreusin-trihydroxzbenzoate.

3. Low Temperature Vacuum Drying

Low temperature vacuum drying is an adaptation of lyophilization that facilitates sterile preparation of dry solid parenteral formulations from certain violatile solvents particularly low molecular weight alcohols. Compounds that are soluble in mixed solvent systems, but undergo hydrolysis on long-term storage in water or ethanol are appropriate candidates, i.e., BCNU, CCNU, etc. However, certain additives might be required to optimize pharmaceutical properties by: (a) increasing powder mass; (b) improving solubility by addition of complexing agents; or (c) enhancing stability by inclusion of antioxidants or buffers. Addition of citrate buffer to the BCNU formulation provided improved solution stability after reconstitution with ethanol and further dilution with nine volumes sterile water. The pH was adjusted to the optimum for nitrosourea stability in aqueous solution (4.0-4.4). Inclusion of polyethylene glycol 1500 or 4000 in the vacuum dried formulation of bruceantin enhanced stability and improved precision of the weight variation determinations.

Low temperature vacuum drying was also adapted to the formulation of NSC-95466, PCNU. A 200 mg vial was prepared from acetone-ethanol 7:3. The dried product is dissolved in dimethylacetamide and then successively diluted with propylene glycol and Sodium Chloride Injection. The formulation exhibits solubility and stability adequate for administration as a four hour infusion.

4. Design of Dosage Forms for Intrathecal Use

Recently, several reports have indicated that pharmaceutical excipients may influence toxicity of antineoplastic agents administered intrathecally. Therefore, the pharmaceutical properties of methotrexate, cytarabine and hydrocortisone succinate were evaluated in three commonly used vehicles: Sodium Chloride Injection, USP; Lactated Ringer's Injection, USP; and Elliott's B solution. Elliott's B solution, an artificial cerebrospinal fluid, has a buffer capacity 20-40 fold higher than Lactated Ringer's or Sodium Chloride Injection and was the only vehicle to markedly influence the pH of drug containing solutions. The pH of methotrexate and cytarabine 1-2.5 mg/ml in Lactated Ringer's and Sodium Chloride Injection were respectively about 8.3 and about 5.6, but between 7.2 and 7.6 in Elliott's B solution. The osmolarity of all drug solutions was between 257 and 305 mOsm/kg and within 10% of human CSF. The chemical and physical stability of methotrexate and cytarabine was not influenced by the choice of vehicle. However, decomposition of hydrocortisone sodium succinate was accelerated in Elliott's B solution. The buffer capacity of the steroid formulation at 1 mg/ml of hydrocortisone was exceeded by Elliott's B solution and the pH (7.4-7.6) outside the range of optimal solution stability (pH ∿ 6.9). At 30°C the useful life in solution (≦ 10% decomposition) was twelve hours for Elliott's B solution and 72 hours for the other vehicles.

These studies were presented at the Eleventh Annual Clinical Midyear Meeting of the American Society of Hospital Pharmacists Meeting in December, 1976 (J. Cradock). The intrathecal route of administration also presents some unique problems to the formulator of parenteral products. A literature survey was conducted of formulations administered by this route to develop some guidelines for use by the product development pharmacist. A report was presented at the Annual Meeting of the Parenteral Drug Association in November, 1976 (J. Cradock).

About four other projects were investigated during the past year. These projects involved routine formulation, solubility and stability evaluations of several new agents.

Publications:

1. Rahman, A., Cradock, J. C. and Davignon, J. P.: Dissolution and Absorption of the Antineoplastic Agent Ellipticine. J. Pharm. Sci., in press.

SUMMARY REPORT

ASSOCIATE DIRECTOR FOR CLINICAL ONCOLOGY

DIVISION OF CANCER TREATMENT

NATIONAL CANCER INSTITUTE

July 1, 1976 - September 30, 1977

The Clinical Oncology Program comprises six intramural Branches concerned with the treatment and investigation of patients with cancer, and incorporates the disciplines of surgery, radiation, medical oncology, and pediatric oncology. Primary emphasis is placed on combined modality treatment of malignancy utilizing surgery, plus adjuvant chemotherapy, radiotherapy, and immunotherapy for patients with localized or regional disease, and radiation, combination chemotherapy and immunotherapy in selected patients with more advanced cancer. The treatment programs are organ-site oriented, and involve a wide variety of malignant conditions, including leukemias, malignant lymphomas, cancer of the breast, ovary, pancreas, lung, colon, stomach, as well as malignant melanoma, sarcomas of bone and soft tissue, and a variety of childhood malignancies. Treatment protocols are regularly reviewed by the NCI Clinical Research Subpanel and are coordinated through the office of the Associate Director for Clinical Oncology. Statistical support for clinical trial design, data compilation and analysis are provided by the Biostatistics and Data Management Section, under the purview of the Associate Director for Clinical Oncology.

This year marks the completion of the first year of a new Branch, Clinical Pharmacology, under Dr. Bruce Chabner. This Branch was created in July, 1976 to conduct clinical and laboratory studies in the pharmacology of antineoplastic agents. The Branch incorporates elements of the Medicine Branch and the Laboratory of Chemical Pharmacology which was relocated in Building 37, and now facilitates collaborative clinical studies with all COP Branches.

Laboratory and clinical research related to the clinical programs are undertaken in each Branch. These range widely in scope, from bedside studies of cancer diagnosis, staging, and drug pharmacology, to basic molecular biology of oncogenic viruses, tumor immunology, endocrinology, and cellular kinetics. These investigations are undertaken by senior staff physicians who have developed a wide variety of laboratory skills; they form a vital link in the provision of both excellent medical patient care and the performance of relevant laboratory studies. There is close cooperation and collaboration among the clinical Branches, as well as with many other Departments and Institutes within the NIH.

Three Branches, Radiation Oncology, Pediatric Oncology, and Medicine have undergone extramural site visits under the auspices of the DCT Board of Scientific Counselors. Site visits of the remaining Branches are scheduled in the coming 2 years. This form of peer review has provided major assistance in assessing progress and identifying priorities in the COP. Some of the changes and new plans in this year's Annual Report reflect recommendations of the site visit committees.

The Clinical Oncology Program has developed a nationally recognized Oncology Fellowship, accredited for eligibility by the Medicine and Pediatric Subspecialty Boards. Clinical Associates (fellows) are assigned to the Branch, and spend a full year with major clinical responsibilities, with a three-month elective in another area; the second year provides experience in the laboratory, with maintenance of some clinical responsibilities. Selected candidates may remain for a third year of continued investigation. In addition to the post-graduate program, clinical electives within the program are available for medical students.

Program Accomplishments

Office of the Associate Director for Clinical Oncology and Clinical Director

This office is responsible for administrative support for the Clinical Oncology Program, and coordinates personnel, equipment, and space allocation. In addition, the dual responsibility of the Associate Director for Clinical Oncology/Clinical Director extends to all clinical activities of the NCI, including protocol review, patient recruitment, patient care, bed allocation, and coordination of education and training of students, nurses, and Clinical Associates. A critical function is the facilitation of interdisciplinary collaborative research between the clinical Branches, the laboratory, and the related programs within the Institute. Extramural responsibilities extend to coordination of the clinical and resource contracts, organization of relevant "state of the art" seminars and conferences, and clinical consultation for a large number of inquiries from patients and physicians around the country.

This year, several major investigative areas were developed from the Associate Director's Office: 1) a collaborative project in treatment of cancer of the bilharzial bladder was undertaken at the Cairo Cancer Institute, supported by PL 480 funds. Protocols in preoperative radiotherapy, surgery, and phase II chemotherapy were developed and implemented. This resource will provide rapid phase II data in bladder cancer. A COP Clinical Associate will be assigned to Cairo this year to assist and facilitate other collaborative projects with NCI; 2) Hepatitis B is associated with primary liver cancer. The development of an effective vaccine by NIAID/BOB has led to a collaborative effort coordinated by this office to establish a vaccine field trial to test the possible etiological association. A recent workshop co-sponsored by FIC, NCI, NIAID and BOB has affirmed the potential utility of a vaccine trial. Areas of needed support and procedural plans have now been established, and inter-institute collaboration will actively continue; 3) Investigative interest in the etiology and treatment of Burkitt's lymphoma is coordinated by the Associate Director. This year field studies in an apparent Eastern Pennsylvania cluster were initiated. The management of Burkitt's lymphoma in relapse using high dose chemotherapy and bone marrow autografting shows considerable promise, with three long term survivors of 18 treated patients; (4) A tumor markers program was defined in conjunction with DTP, to facilitate specimen collection. Space and personnel were identified, and new protocols were developed to study biochemical markers in cancer of the breast, ovary, lung, and colon; 5) Collaborative studies with the Chester Beatty Research Institute in London are being initiated under the auspices of the AO/COP. These plans include development of phase I-II trials of pentamethylmelamine, with coordinated testing in human tumor xenografts. Other areas of potential clinical importance will be supported: drug-drug interaction; role of DNA repair in drug resistance; and rescue schemes to protect normal tissues from cytotoxic injury.

Biostatistics and Data Management Section

This section provides program support in assisting with trial design, data collection (with computerized storage and retrieval systems) data analysis, and research in relevant statistical methodology and mathematical models. A major accomplishment of this section has been the elaboration of the "fraction kill hypothesis" and its clinical implication by Drs. Norton and Simon. Simply stated, the theory holds that solid tumors grow according to Gompertzian kinetics. Treated with chemotherapy, relative resistance develops in small tumors because of first-order kinetic cell kill. Although the growth fraction is large in smaller tumors, the number of cells killed is smaller than when the tumor is growing exponentially. Thus, in treating micrometastatic tumors, more intensive chemotherapy is required. This hypothesis is supported by clinical observation and animal experiments. Several clinical trials have been designed to test the theory prospectively.

Clinical Branches

The following represent a brief notation of some of the major accomplishments of the COP during the past year. Further details can be found in the individual project reports.

Clinical Pharmacology Branch

1. The discovery that free radical formation and lipid peroxidation leads to adriamycin cardiotoxicity, and that this effect can be prevented by vitamin E.

2. Methotrexate can be safely administered in high dose using routine drug level monitoring to guide leukovorin rescue.

3. Discovery that mixed function oxidase activity in bronchial Clara cells will activate furan-type pulmonary toxins; this observation has implications for therapy and carcinogenesis.

4. Observation of an excess of phosphodiesterase activity in tumor cells, which may have multiple physiologic functions in regulation of cell growth.

5. The discovery of marked differences in "tight-binding" enzyme inhibitors (e.g. methotrexate) in intact cells versus cell-free preparations.

Medicine Branch

1. Superiority of HEXA-CAF over L-PAM in management of ovarian cancer.
2. Detection of estrogen receptors in malignant melanoma tissue.
3. Elucidation of carriers of methotrexate transport.
4. Development of a prolactin-dependent rat mammary cancer cell-line.
5. Establishment of curability of nodular mixed non-Hodgkin's lymphomas.

NCI-VA Medical Oncology Branch

1. Intensive chemotherapy causes 52% complete responses in oat cell cancer.
2. High-dose methotrexate is ineffective in non-oat cell cancer.
3. Beneficial responses to chemotherapy and electron-beam radiation therapy in patients with Sezary syndrome and mycosis fungoides.
4. Successful mapping of mouse genes responsible for murine leukemia and primate C-type virus replication.

Pediatric Oncology Branch

1. Discovery that bone marrow autografts accelerate hemopoietic recovery in patients treated with intensive chemotherapy.
2. Determination that American patients with Burkitt's lymphoma have similar therapeutic results (54% long term survival) to their African counterparts.
3. Discovery of neurological sequellae of prophylactic cranial radiation in patients with acute leukemias.
4. Observation that probenecid retards the egress of methotrexate from the cerebrospinal fluid leading to new treatment strategies in brain tumors.
5. Granulocyte transfusions were determined to be life-saving in patients with prolonged marrow failure, but did not alter the clinical course of patients whose marrow recovered rapidly.
6. The observation of minor deletions of viral sequences in non-leukemic AKR mice, and the discovery of more viral genomes in "high" versus "low" leukemic animals.
7. Glucocorticoid receptors are decreased and adenosine deaminase in T cell leukemias; these changes have prognostic implications.

8. The human alpha globin gene has been mapped on chromosome 16 and the beta globin gene on chromosome 11.

Radiation Oncology Branch

1. High remission rates and long term survival in oat cell cancer of the lung have been achieved with combined radiation and chemotherapy.
2. Radiation therapy alone results in long term survival in patients with stage II nodular sclerosing Hodgkin's disease.
3. Adjuvant radiotherapy in sarcomas of the extremity is equivalent to radical surgery in establishing local control.
4. Computerized axial tomography is being limited to radiotherapy treatment planning to facilitate "dynamic" delivery of radiation to tumors.

Surgery Branch

1. Clinical demonstration of the effectiveness of chemotherapy in adjuvant therapy of sarcomas.
2. Local surgery plus radiotherapy is as effective as radical surgery in local control of sarcomas.
3. Human sarcomas express fetal tumor-associated antigens.
4. The demonstration that nutritional deprivation can lead to enhanced tumor cell division in an animal model.
5. Demonstration that thymosin can *in vitro* correct T cell depletion resulting from mediastinal irradiation.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 06308-06 CO

PERIOD COVERED
July 1, 1976 through September 30, 1977

TITLE OF PROJECT (80 characters or less)

Biostatistics and Data Management

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	R. Simon	Head, Biostatistics and Data Management Section	COP, DCT, NCI
OTHER:	L. Norton	Clinical Associate	COP, DCT, NCI
	S. H. Li	Visiting Fellow	COP, DCT, NCI
	M. Kruse	Medical Record Librarian	COP, DCT, NCI
	Y. J. Lee	IPA Consultant	COP, DCT, NCI
	R. W. Makuch	Staff Fellow	COP, DCT, NCI

COOPERATING UNITS (if any)
Clinical Branches COP, DCT, NCI; Cancer Therapy Evaluation Program, DCT, NCI; Immunology Branch, DCBD, NCI

LAB/BRANCH
Clinical Oncology

SECTION
Biostatistics and Data Management Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
4.0	3.0	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The objective of the Biostatistics and Data Management Section is the utilization of statistical methodology and computer technology to maximize knowledge obtained from clinical trials and related research carried out within and on grant or contract to the clinical programs of the Division of Cancer Treatment. The section collaborates in the design and analysis of clinical trials and other experiments, develops data collection procedures and related computer systems, and maintains computer files of collected data. The section also conducts biometric research in support of the above goals. A list of projects in which the section is actively involved follows.

1. Participation in the Design and Conduct of Clinical Research

The section provides statistical consultation for the design of therapeutic clinical trials and related research performed within the Clinical Oncology Program. Design and review of major extramural clinical trials to be performed under auspices of the Cancer Therapy Evaluation Program is also conducted. The section also develops and maintains computer systems of detailed patient data, and serves as the statistical coordinating center for design, randomization, data collection and analysis for intramural clinical trials and for selected collaborative extramural clinical trials (e.g. trials of the ovarian cancer study group).

2. Statistical Analysis

The section provides statistical consultation and collaboration in the analysis of clinical studies. Collaborations this year have included: (1) comparison of CAF to CMF in the treatment of metastatic breast cancer; (2) comparison of CAF to DAV-CMF alternation in the treatment of metastatic breast cancer; (3) comparison of CAF, CFP, and CA in the treatment of metastatic breast cancer; (4) comparison of adrenalectomy to combination chemotherapy in the treatment of metastatic breast cancer; (5) evaluation of clinical prognostic factors in patients with osteogenic sarcoma; (6) evaluation of high dose methotrexate with leukovorin rescue in the adjuvant treatment of osteogenic sarcoma; (7) evaluation of the immunological and anti-tumor effect of BCG in the adjuvant treatment of osteogenic sarcoma, and evaluation of immunological parameters as potential predictors of disease recurrence in such patients; (8) comparison of L-PAM to HEXA-CAF in the treatment of unresectable ovarian carcinoma; (9) evaluation of cyclic combination chemotherapy in the treatment of unresectable small cell cancer of the lung; (10) evaluation of factors associated with the development of cardiac metastases in lung cancer; (11) evaluation of the *in vivo* effects of thymosin in patients with small cell cancer of the lung receiving chemotherapy; (12) evaluation of the effects of *in vitro* thymosin on T-cell levels in cancer patients receiving radiation therapy; (13) evaluation of 5FU and CCNU in the treatment of metastatic prostatic cancer; (14) evaluation of CxT methotrexate via a subcutaneous reservoir for the intraventricular chemotherapy of CNS neoplasms; (15) evaluation of BCG immunotherapy in the maintenance treatment of patients with acute lymphatic leukemia; (16) analysis of the non-randomness of cytogenetic abnormalities and the effect of age on cytogenetic abnormalities in patients with acute leukemia; (17) evaluation of changes in the incidence of episodes of gram positive and gram negative bacterial sepsis in pediatric oncology patients; (18) evaluation of prognostic factors and long term follow-up results in the treatment of advanced Hodgkin's disease; (19) evaluation of Velban for the treatment of recurrent Hodgkin's disease; (20) evaluation of MOPP as a reinduction for patients with recurrent Hodgkin's disease; (21) comparison of pathological classification systems for non-Hodgkin's lymphomas; (22) evaluation of the prognostic importance of change in histology for patients with non-Hodgkin's lymphoma; (23) review of all patients treated at NCI for non-Hodgkin's lymphoma with respect to pathological classification, clinical characteristics, and clinical course; (24) evaluation of the importance of histological cell type on prognosis in the nodular non-Hodgkin's lymphomas; (25) evaluation of the effect of prior radiotherapy on tolerance and response to chemotherapy of patients with non-Hodgkin's lymphoma; (26) analysis of the effect of methotrexate and vincristine dose and schedule of administration on intra-cellular methotrexate concentration and survival of mice with L-1210 murine leukemia; (27) evaluation of prognostic factors in patients with Ewing's sarcoma; (28) evaluation of immunotherapy with neuraminidase-treated tumor cell vaccine in mice bearing established methylcholanthrene sarcoma; (29) evaluation of factors predicting hematological recovery following bone marrow transplantation in dogs; (30) evaluation of the effect of lymphoma cells on the mixed leukocyte culture response for dogs; (31) evaluation of the relationship between number of steroid receptor sites per cell in lymphocytes to cell surface and MLC characteristics; (32) evaluation of the effects of wbc collection methods and cell storage on post transfusion wbc counts in leukopenic dogs; (33) evaluation of the effect of methotrexate with leukovorin rescue and of cis-platinum of in vitro CFU assay; (34) analysis of the influence of HL-A and ABO antigens and prior transfusion history on platelet increments following tranfusion; (35) analysis of liver function test abnormalities following chemotherapy in patients with hepatitis associated antigen; (36) analysis of the effect of BCG and prior tumor exposure on the growth curves of transplanted melanomas in mice; (37) comparison of the effects of suctioning techniques on arterial blood gas alterations; (38) analysis of statistical considerations in the determination of ventricular volume from echocardiographic and angiographic data.

3. Tumor Registry

The section maintains a tumor registry for patients admitted to the Clinical Center, and performs regular follow-up for all patients. Inquiries from outside tumor registries are handled by the section.

4. Modeling and Biometric Research

In 1975 the section initiated the development of mathematical model to describe the growth curve of individual tumors and the manner in which such growth curves are altered by the administration of cytotoxic agents. This model is empirically justified and is not based upon hypothetical assumptions about biological mechanisms, nor does it require the estimation of unmeasurable biological parameters. The objective of this project was to develop a simple model for predicting effective schedules and doses and effective ways of combining drugs and modalities in the treatment of solid tumors. Initial experiments for testing this model in the B16 melanoma, 13762 mammary adenocarcinoma, and in Martin's spontaneous carcinoma have been more than encouraging. In addition, use of this model has clarified that a large body of clinical and laboratory results are not consistent with the usual "log-kill" hypothesis, and has suggested that a thorough re-evaluation of the concept that maintenance and adjuvant chemotherapy regimens need not be intensive is in order. The model also suggests a new approach to the design and analysis of experiments for the screening of anti-tumor agents in animals that may considerably increase the efficiency of such experiments.

Other major biometric research projects include: (1) investigation of sequential termination and treatment assignment rules for the development of clinical trials which require fewer total patients and assign fewer patients the inferior treatment than is accomplished by traditional designs; (2) investigation of the effects of stratified randomization on the power and significance level of statistical tests; (3) development of methods of estimating sample sizes required for randomized chronic disease studies utilizing prognostic factor information; (4) determination of methods of adjusting for "length bias" in etiologic studies; (5) evaluation of the importance of the "continuity correction" in the use of the Mantel-Haenszel test for several contingency tables; (6) determination of methods for sample size estimation for repeated measure experiments.

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 06402-07 CP

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)
Tumor Growth Kinetics and Chemotherapy

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

P.I.:	Stanley E. Shackney, M.D., Senior Investigator	CPB,DCT,NCI
OTHER:	Gregory McCormack, M.D., Clinical Associate	NCI-VA MOB,DCT,NCI

COOPERATING UNITS (if any)
NCI/VA MOB
Western Lymphoma Group
LCP,DCT,NCI (Dr. Zaharko)

LAB/BRANCH
Clinical Pharmacology

SECTION

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS: 4	PROFESSIONAL: 2	OTHER: 2

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☒ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Computer modelling studies of drug effects in vitro are in progress. In vitro studies on cytosine arabinoside, adriamycin, colcemid and cytochalasin B, and two dose schedule optimization studies employing ara-C and hydroxyurea are in progress. In vivo studies on the effects of hydroxyurea in mouse bone marrow are also in progress. Preliminary observations suggest that recruitment predominates over synchronization during recovery. Flow microfluorometry (FMF) studies have established the T lymphocyte as a useful diploid reference standard. FMF studies on the non-Hodgkin's lymphomas and on the ploidy of a variety of human solid tumors are ongoing. Tumor doubling times were measured in small cell carcinoma of the lung. A review of tumor doubling times in human solid tumors in relation to responsiveness to therapy and curability was completed.

Project Description:

1. Theoretical and methodological analysis

1.1 Computer modelling studies

A detailed analysis of the kinetics of human AML and malignant melanoma was published in the Journal of Theoretical Biology in May, 1977.

Limited modelling studies of the kinetics of drug perturbation in vitro were undertaken this year. These studies will be expanded considerably in the summer and fall of 1977.

2. Experimental Studies

2.1 Drug studies of sarcoma 180 in vitro

2.1.1 Hydroxyurea

a paper describing the kinetic effects of hydroxyurea in sarcoma 180 in vitro has been accepted for publication in Cancer Research.

Major Findings:

i.) Drug induced changes in radioautographic labelling intensity reflect true changes in the rate of DNA synthesis, and are not attributable to thymidine pool size effects.

ii.) The spectrum of drug concentration dependent lethal and sublethal effects of hydroxyurea is more consistent with the concept of graded DNA-synthesis-rate-dependent lethality than with the all or none concept of cycle-stage-specificity. That is, drug lethality varies directly with DNA synthesis rate being greater in cells that synthesize DNA more rapidly.

2.1.2 Cytosine arabinoside

Studies on the kinetic effects of cytosine arabinoside in vitro, as a function of drug concentration and exposure duration, were completed this year.

Major Findings:

i.) At drug concentrations of ara-C that are equally lethal with hydroxyurea, the depression in the rate of DNA synthesis in surviving cells is greater, and time to recovery is more prolonged in the ara-C treated cells.

ii.) In contrast to hydroxyurea, increasing drug concentrations and increasing drug exposure duration both led to increased cell killing.

iii.) The classification of drugs merely as cycle-stage specific vs. non-stage specific is clearly inadequate for conveying the kinetic information that is likely to be most useful in therapy planning.

2.1.3 Schedule optimization studies

The time to recovery of maximum rate of DNA synthesis after single doses (5-50mM) by hydroxyurea (6-10 hours) differs from that following $5\text{-}50\times10^{-6}$M ara-c (12-16 hours). Studies are underway to determine the optimum time for a second dose of drug in relation to the recovery in DNA synthesis rate. Preliminary observations indicate that following an initial dose of hydroxyurea, both ara-C and a second dose of hydroxyurea produce maximum cell kills at 8-10 hours. Studies utilizing ara-c as the first drug are in progress. At present the data suggest that:

i.) DNA synthesis rate (i.e., labelling intensity of individual cells) would appear to be a more important determinant of hydroxyurea and ara-C lethality than the fraction of dividing cells, as measured by labelling index.

ii.) Recovery kinetics are more important than steady state cell cycle kinetics in planning multidose regimens. Differences in time to recovery between normal and neoplastic tissues may be exploited therapeutically, whichever tissue recovers first. Future studies will examine adriamycin, colcemid, cytochalisin B and other drugs in multidrug, multidose schedules.

2.1.4 Adriamycin studies

Our studies in a patient with neuroblastoma (see #3.2, below), as well as other recently reported clinical kinetic studies employing cytoxan and adriamycin indicate that early stimulation of DNA synthesis may occur following drug administration. This may be of importance in the scheduling of multiple drugs. Radioautographic studies, FMF, and cell cloning studies are in progress in sarcoma 180 *in vitro* to explore this phenomenon in relation to adriamycin administration.

2.2 Experimental mouse bone marrow studies *in vivo*

With Dr. McCormack, the kinetics of post-hydroxyurea recovery in mouse with bone marrow were investigated; folowing initial exposure to hydroxyurea, animals were given ^{3}HTdR and sacrificed at intervals. Radioautographs of transverse marrow sections showed immediate inhibition of DNA synthesis in the rapidly proliferating subendosteal cells. The recovery phase occurred between 12 and 24 hours, involving cells throughout the marrow, including the central differentiating zone. By 48 hours the subendosteal proliferative pattern was reestablished. These are qualitative, preliminary observations. Because of the technically poor quality of many of the tissue sections, quantitative kinetic analysis will be performed on slides obtained from a repeat study, now in progress. Our findings to date suggest that:

i.) Recruitment is a more prominent feature of post-hydroxyurea recovery than cell synchronization.

ii.) The differentiating cells in the central region of the marrow may be capable of at least limited participation in the recuritment phenomenon. Future studies will examine multidose schedules.

2.3 Flow microflourometry - experimental studies

2.3.1 Colcemid and cytochalasin B studies

Since critical normal host tissues proliferate more rapidly than most human tumors, they are likely to be more amenable to mitotic collection. Cells that are reversibly arrested in mitoses may be protected from the effects of drugs that act elsewhere in the cell cycle. Published reports indicate synergism between colcemid and cytochalasin B in producing mitotic arrest. Mitotic arrest can be studied most conveniently by FMF methods.

In preliminary C x T studies employing colcemid and cytochalasin B in sarcoma 180 *in vitro*, it was found that even low concentrations (10^{-6}-10^{-7} M) of colcemid produce extensive cell fragmentation with prolonged drug exposure. Studies are planned with still lower drug concentrations, in an attempt to find an optimal combination for achieving reversible mitotic arrest for *in vivo* studies.

2.3.2 The T lymphocyte as a diploid reference standard for human ploidy analsys of tumors by FMF

The T lymphocyte was found to be a reliable diploid reference standard for human ploidy studies. Diploidy was measured reproducibly by FMF from one sample to another and from one individual to another. This was true for both the mithramycin and acriflavine-Feulgen DNA staining systems. Fixed T lymphocytes were stable for 3 months or more. These findings are being prepared for publication.

2.3.3 Glutaraldehyde will be evaluated this year as a fixative to replace alcohol or formalin. Glutaraldehyde may be superior for multiparameter studies.

2.3.4 Protein content will be considered a a third parameter for study in the anlysis of human tumors. Preliminary studies will be carried out in sarcoma 180.

2.3.5 Preliminary studies with cell sorting will be undertaken this year. The main objectives initially will be to:

i) Obtain histologic correlations with FMF patterns, and

ii) Perform kinetic studies on prelabelled cells (^{3}HTdR) sorted by DNA content and coulter volume.

3. Clinical studies

3.1 Sezary's syndrome

3.11 Pulse labelling studies

In collaboration with Dr. Bunn, two patients with Sezary's syndrome were given pulse ^{3}HTdR I.V., and serial blood and skin biopsy samples were obtained over a 14-day period. Radiographs were prepared, and normal and neoplastic mitoses and interphase cells were evaluated for label. These studies will be completed by mid-July.

Major findings:

i) Normal skin cells labeled within 2-6 hours in both patients. Peak labelling in one patient's Sezary's cells in the skin did not occur until 24 hours after pulse. In this patient peak blood labelling of Sezary's cells also occurred at 24 hours.

ii) PLM curves in normal skin were similar to those of Sezary's cells in the skin, but the mitotic indexes were much lower in the Sezary's cells than in the skin.

iii) Conclusions: While Sezary's cells do proliferate in the skin, only a small fraction grow as rapidly as normal skin cells. In at least 1 patient, the major sites of cell production were elsewhere, perhaps the lymph nodes.

3.1.2 Sezary's syndrome, long term ^{3}HTdR infusions

In collaboration with Dr. Bunn, two patients were given 20 day ^{3}HTdR infusions, during which serial blood and skin biopsy samples were obtained. Specimens were processed as above. Grain counts are nearing completion in the blood of the first of these patients.

Major findings:

i) Labelled Sezary's cells appeared more heavily labelled in the blood than mature granulocytes. This may mean that there is a small pool of Sezary's cells that is turning over more rapidly than normal myeloid cells.

The processing of slides from the second patient will be undertaken this year.

3.2 Kinetic studies in neuroblastoma during combination chemotherapy

Combined FMF and radioautographic studies in the marrow of a patient with neuroblastoma were completed this year.

Major findings:

i) Combination therapy with vincristine, cytoxan, and adriamycin produces complex sequences of kinetic events in human marrow, including mitotic arrest (VCR), stimulation of DNA synthesis, and a round of abortive DNA synthesis with cell death in S. These findings were published as an ASCO abstract this year and a full manuscript is in preparation.

3.3 Tumor Doubling times in small cell carcinoma of the lung.

In collaboration with members of the NCI/VA MOB, the x-rays of 144 untreated patients with small cell carcinoma were received for measurable lesions. Serial doubling time measurements were made in 12 patients whose films were considered suitable. Doubling time ranged from 25 to 155 days with a mean of 90 days. This is considerably longer than expected from isolated reports of tumor doubling times in small cell cancer found in the literature.

3.4 A review of doubling times in human solid tumors in relation to therapeutic responsiveness.

With Dr. McCormack, a review of human solid tumor doubling times was carried out in relation to therapeutic responsiveness and curability. Generally, it was observed that rapidly growing tumors are responsive and curable, while slowly growing tumors respond poorly to therapy. A strategy for improving the treatment of rapidly growing tumors was developed, which depends on the distinction between early and late tumor recurrences for its implementation.

A paper describing the application of this strategy in small cell carcinoma of the lung was presented at the Airley House Conference on Lung Cancer this year.

3.5 FMF - clinical studies.

3.5.1 Non-Hodgkin's lymphomas

Over 20 specimens from patients with non-Hodgkin's lymphomas were analyzed by dual parameter FMF, in collaboration with Dr. Lincoln.

Major findings:

i) Histiocytic lymphomas and poorly differentiated lymphomas are aneuploid, while well differentiated lymphomas and chronic lymphocyte leukemia are deploid.

ii) The large cell lymphomas proliferate more rapidly than the small cell lymphomas.

III) Within a given lymphoma, the largest G_1 cells are the most rapidly proliferating.

3.5.2 Ploidy of human tumors

Over 30 specimens from a variety of human tumors have been analyzed to date by dual parameter FMF, as well as normal tissues. Normal tissues have all been diploid, but occasional unusual non-neoplastic specimens (1 granulomatous process and 1 atypical reactive hyperplasia) have been hyperdiploid. Most solid tumors have been aneuploid, with some ovarian Cas showing extreme hypodiploidy. Many of the specimens obtained at NIH have also been studied in parallel by standard cytogenetic methods to compare results of both methods. These studies will continue throughout the coming year.

3.5.3 The main thrust of new studies will be the serial monitoring of drug effects in man using FMF and autoradiographic methods.

4. Instrumentation

4.1 Scheduled for delivery later this year by the Los Alamos Group are a software package for quantitative DNA histogram analysis and improved software display and analysis programs. A doublet discriminator has recently been installed. It is possible that an improved low noise counter orifice may be available later in the year. All of these will require testing and evaluation during the coming year.

4.2 Development and testing of a grain counter which will also permit DNA measurements on the same cells by electronic image analysis techniques will be undertaken during the coming year.

Publications:

Ford, S.S., and Shackney, S.E., Lethal and sublethal effects of Hydroxyurea in Relation to drug Concentration and Drug Exposure Duration in Sarcoma 180 *in vitro*. *Cancer Res*. In Press.

Bunn, P.A., Shackney, S.E., and Ford, S.S. Effects of Colcemid on Hematopoiesis in the mouse. *J. Clin. Invest*. 58: 1280-1285, 1976

Bunn, P.A., Ford, S.S. and Shackney, S.E. Rapid Cell and Grain Counting. *J. Nat. Cancer Inst.* In Press.

Shackney, S.E., Erickson, B., and Lincoln, T.L., Preliminary observations in the non-Hodgkin's lymphomas using multi-parameter flow analysis. *Proceedings of the Third International Symposium on Pulse Cytophotometry*. Vienna, 1977. In Press.

Shackney, S.E., Cohen, M., Ihde, D., Bunn, P.A. and Minna, J. The application of principles of Cell Kinetics in the Treatment of Small Cell Carcinoma of the Lung. Proceedings of the 2nd Airlie House Conference on Lung Cancer, Airlie, Virginia, 1977. In press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 06510-01 CP

PERIOD COVERED July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Mechanisms of Tissue Toxicity Caused By Adriamycin

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Charles Myers, M.D.	Senior Investigator	CPB,DCT,NCI
OTHER:	Harold Londer, M.D.	Clinical Associate	CPB,DCT,NCI
	James Doroshow, M.D.	Clinical Associate	CPB,DCT,NCI
	Gershon Locker, M.D.	Clinical Associate	CPB,DCT,NCI
	John Baldinger,	2nd yr. Med. Student, Vanderbilt University School of Medicine and CO-STEP Participant, Summer of 1977	

COOPERATING UNITS (if any)

Robert Young, M.D.	Medicine Branch	DCT,NCI
William McGuire, M.D.	Medicine Branch	DCT,NCI
John Gottdiener, M.D.		NHLBI

LAB/BRANCH Clinical Pharmacology

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NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
5	4	1

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

1. Adriamycin Cardiotoxicity: (Myers, Eliot, Brooks, Locker, Doroshow, McGuire)

This laboratory has recently proposed that adriamycin cardiotoxicity results from free radical generation during drug metabolism. The basic observations supporting this contention have been recently accepted for publication in Science.

We are currently examining the endogenous defenses of cardiac tissue against free radical damage to see what role they might play in the clinical pattern of cardiotoxicity. Finally, we are actively engaged in the design of a clinical trial testing the ability of various free radical scavengers such as Vitamin E to ablate adriamycin cardiac toxicity.

PHS-6040
(Rev. 10-76)

1. Adriamycin Toxicity

Doctors Doroshow and Locker are examining the role of free radicals in the etiology of adriamycin toxicity. Studies are underway to evaluate the effect of manipulations of the seleno-enzyme glutathione peroxidase on murine cardiac and hepatic lesions due to adriamycin. These have involved extensive studies on the interrelationship between nutritional selenium and the cardiac activity of glutathione peroxidase, as well as the direct effect of adriamycin on peroxidase activity. We have found definite evidence of enhanced adriamycin toxicity in peroxidase deficient animals.

We are also investigating the role of a variety of thiol compounds both *in vivo* and *in vitro* as agents capable of protecting against adriamycin toxicity. In addition to these current projects, we have concluded studies on the role of membrane lipid saturation, alpha tocopherol content, and peroxidative damage to tissues caused by adriamycin.

2. Adriamycin Pharmacokinetics

Studies are underway to examine the relationship between clinical perturbations of hepatic or renal function and adriamycin disappearance and metabolism in our patients. These investigations will also explore the effect of chemotherapeutic drug combinations containing adriamycin on adriamycin pharmacokinetics.

3. Specific Sites of Cardiac Biochemical damage following adriamycin

With Helen Eliot, we are examining the specific omponents of the cardiac cell damage following adriamycin. Given the working hypothesis that the damage proceeds via a free radical mechanism, it is possible to predict that such endogenous compounds as coenzyme Q10 and membrane phospholipids should be destroyed to yield identifiable oxidized products. We are in the process of studying these changes as they might provide important insight as to why the lesions can progress for months after the last dose of adriamycin.

Publications:

Myers, C.E., McGuire, W.P., Liss, R.H., Ifron, I., Grotzinger, K., Young, R.C.: Adriamycin: The Role of Lipid Peroxidation in Cardiac Toxicity and Tumor Response, *Science*, in press.

Myers, C.E., McGuire, W.P., and Young, R.C.: Adriamycin: Amelioration of Toxicity by α-Tocopherol. *Cancer Treatment Reports*, Vol. 60, No. 7: 961-962, July, 1976.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 06511-01 CP

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Molecular Pharmacology of Cyclic Nucleotides

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

P.I.: Dr. Yung-Pin Liu, Cancer Expert CPB,DCT,NCI
Dr. Ying Ming Lin, IPA, St. Jude Childrens Research Hospital, Tennessee CPB,DCT,NCI

OTHER: Dr. Vernon Wong - Dept. of Ophthalmology, Georgetown University
Dr. Gopal Krishna - Lab. of Chemical Pharmacology-NHLBI
Dr. Gerald Chader - Lab. of Vision Research NEI
Dr. M.C. Lin - Lab. of Nutrition and Endocrinology-NIAMDD

Student Scientist: Philip Gilly - Johns Hopkins University
Stay-In-School Program: Howard Schwartz - University of Maryland

COOPERATING UNITS (if any)

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3	3	0

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Cyclic AMP plays an important role in the regulation of hormonal actions and of cell growth. Cyclic nucleotide phosphodiesterase (PDE), which catalyzes the hydrolysis of cyclic AMP, exists in several molecular forms in many tissues. A dependent form of PDE requires a Ca^{++} binding protein to reach maximal activity through the formation of Ca^{++} -activator-enzyme complex. By the selective inhibition or activation of the PDE isozymes, it may be possible to alter the course of diseases characterized by an abnormal metabolism of cyclic nucleotides.

Our major project has been to characterize the phosphodiesterase found in human malignant cells.

In the homogenates of human lymphoblastic leukemic cell line (CEM), 15% of the total PDE activity is in the activator dependent form. The 12,000 xg supernatant shows three distinct PDE activity peaks by DEAE-cellulose column. One of these enzymes was activated by activator purified from either CEM cells or from the ovine brain.

In subcellular fractions of the bovine retina, activator-dependent PDE makes up 20% of the total PDE activity in the 105,000 xg supernatant. The nuclear, mitochondrial, and microsomal fractions contain the independent molecular form of PDE. Bovine rod outer segment and lens also appear to have only the independent form. However, the protein activator of PDE was detected in all fractions and tissues mentioned above. It is possible that the abundant activator may play other physiological roles in these fractions and tissues which lack activator dependent PDE.

We have also shown that another physiological role of the activator obtained from CEM and bovine retina may concern its ability to activate the activator-deficient adenylate cyclase of rat brain.

A considerable portion of our current work is being devoted to the characterization of a heat stable, non-dialyzable inhibitor of PDE which was isolated from bovine retina, kidney, and liver by DEAE-cellulose chromotography. The inhibitor appears to be a protein since it was susceptible to trypsin digestion but not to DNase or RNase. Its molecular weight is greater than 200,000. The inhibitor obtained from kidney and liver specificallycounteracts the activation of PDE by the activator. The bovine retina inhibitor inactivates about 60% and 90% of the total independent and dependent PDE activity in retina and CEM supernatants, respectively. Studies on the physical and chemical properties of the inhibitor and the mechanism of the inhibition are currently in progress.

After birth, the level of cyclic GMP in the retinas of a dog with inherited retinal degeneration rises to a peak that is ten times higher than in normal dogs, while the level of cyclic AMP remains relatively constant in both. Homogenates of normal and dystrophic dog retinas contain an excess of endogenous PDE activator and have approximately equal quantities of dependent and independent PDE. The relationship among cyclic GMP, PDE, and the activator in normal and dytrophic dog retinas is under study.

A simple purification procedure with high yield is being developed for the purification of activator from CEM and other tissues. The physical and chemical characterization of CEM activator will then be undertaken.

Publications:

Liu, Y.P., and Speer, R.J.: The carboxyl- and amino-terminal amino acids and other properties of L-asparaginase from *Escherichia Coli*. *J. Form. Med. Assoc.* 75: 168, 1976.

Liu, Y.P., and Cheung, W.Y.: Cyclic 3',5'-nucleotide phosphodiesterase. Ca^{++} confers more helical conformation to the protein activator. *J. Biol. Chem.* 251: 4193, 1976.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 06512-01 CP

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Drug Disposition, Metabolism, and Toxicity

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

P.I.:	Michael R. Boyd, M.D., Ph.D., Senior Investigator	CPB,DCT,NCI
OTHER:	Charles N. Statham, Ph.D. Research Associate, Pharmacology-Toxicology Research Assoc. Program, NIGMS	CPB,DCT,NCI
	John S. Dutcher, Ph.D., IPA-Research Associate	CPB,DCT,NCI (7/1/77)
	Alan R. Buckpitt, Ph.D., NIH Postdoctoral Fellow	CPB,DCT,NCI (7/1/77)
	Nancy S. Longo, M.S., Pharmacologist	CPB,DCT,NCI (7/1/77)
	Roy Jones, M.D., Ph.D., Clinical Associate	CPB,DCT,NCI (7/1/77)
	Henry A. Sasame, Ph.D., Chemist, Lab. of Chemical Pharmacology	NHLBI
	Ronald B. Franklin, Visiting Fellow	NHLBI (Will depart NIH on 8/1/77)

COOPERATING UNITS (if any)

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
8	7-3/4	1/4

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This laboratory has underway three major program areas. In accord with the needs and goals of the Clinical Pharmacology Branch, we are developing a program in analytical pharmacology to provide extensive capabilities for study of both qualitative and quantitative aspects of the metabolism and disposition of cancer chemotherapeutic agents in experimental systems, as well as in man in a clinical setting. In recent months, we have extensively evaluated, and have subsequently recommended for purchase, a variety of essential equipment for these studies. In addition, new laboratory personnel have been recruited to provide an optimal degree of diversity of training and experience appropriate for the group's efforts in this program. Our entire laboratory staff participate in various aspects of this program area.

A second major area of interest of our group is the study of mechanisms of chemically-induced diseases of the lung. The studies have centered on pulmonary toxicities of both " model" pulmonary toxins in animals, as well as agents known to produce lung disease in man. These studies have demonstrated that drugs and other chemicals can produce lung lesions in three ways:

a) by direct alkylation of lung tissue, b) by alkylation following in situ metabolism of the parent compound to a more reactive derivative, and c) by reaction of an initially-formed free-radical metabolite with molecular oxygen to form toxic oxygen species, such as superoxide. These studies are presently being extended to include the investigation of lung toxicities produced by a variety of cancer chemotherapeutic agents since lung lesions produced by these drugs may sometimes severely limit their therapeutic utility.

In a third major area of endeavor in our laboratory, we are attempting to determine if principles we have established in our studies of chemically-induced pulmonary toxicities can be applied to the rational design, synthesis, and evaluation of new types of chemotherapeutic agents for use against bronchogenic lung cancers.

In the following section, a number of our specific projects currently underway are briefly described.

1. Mechanism of Nitrofurantoin-induced lung disease. (Boyd, Sasame, Statham) Nitrofurantoin (NF), a widely used urinary tract antibiotic, is probably the drug most commonly reported causing serious lung disease in patients. We have developed an animal model for nitrofurantoin-induced pulmonary toxicity and have found that the reaction resembles an exaggerated form of pulmonary oxygen toxicity. An oxygen-enriched atmosphere synergizes the toxic response to NF. In vitro studies indicate that the superoxide radical is generated during metabolism of NF by lung enzymes. Moreover, feeding a vitamin E deficient diet, or a diet high in polyunsaturated fat, both increase susceptibility to NF-induced lung lesions. These results suggest that NF causes lung lesions by stimulating the production of superoxide in vivo, and that superoxide may cause the peroxidative destruction of essential lipids in the lung. Further studies of this, and other potential mechanisms of NF-induced lung lesions are underway.

2. Cobalt-induced tissue levels of reduced glutathione. (Boyd, Sasame) We have recently found that administration of the cobaltous ion (as $COCl_2$) ip to a variety of animal species, causes a very striking increase (200-300%) in tissue levels (liver, lung, kidney, ileum) of reduced glutathione (GSH). This effect occurs simultaneously with the previously documented effect of Co++ on hepatic cytochrome P450 levels (30-40% decrease); we have also observed a similar effect on P450 in extrahepatic tissues (lung, kidney, ileum). Other divalent metal ions are much less effective than Co++ in producing the GSH increase. These results indicate that use of cobalt as an experimental tool to implicate cytochrome P450-mediated formation of chemically reactive, toxic drug metabolites, in vivo, may not be valid, since GSH plays an important role in the detoxication of many of these alkylating metabolites. On the other hand, we feel this unique effect of cobalt on tissue GSH levels may provide an important new tool for the study of the regulation of tissue stores of GSH, and intensive investigations are currently underway in this regard.

3. Cellular site of drug-metabolizing enzymes in lung. (Boyd) Recent studies in our laboratory, indicate that nonciliated bronchiolar ("Clara") cells are a major site of cytochrome P450-dependent enzymes in lung. Crucial evidence for this discovery was provided by autoradiographic studies of the location of covalently bound metabolite of 4-ipomeanol in lungs of experimental animals. The toxic metabolite of 4-ipomeanol is formed via a P450-mediated transformation, and the metabolite reacts irreversibly with tissue components at its site of formation.

The finding that Clara cells contain P450-dependent enzymes has important implications for our further understanding of the pathogenesis of chemically-induced lung diseases. Cytotoxic, and/or carcinogenic metabolites may be produced in these cells; the Clara cell may therefore be an especially susceptible target cell for toxins or carcinogens activated by cytochrome P450-dependent systems.

In continuing studies we are attempting to gather further data confirming the metabolic capabilities of the Clara cell both in *in vitro* and *in vivo* systems, and to determine if any other types of lung cells also contain detectable levels of P450-dependent enzymes.

4. Metabolism and Toxicity of 3-Methylfuran. (Boyd, Franklin , Statham) A recent report from the Naval Research Laboratory suggested that a major air pollutant during photochemical smogs in Washington, D.C., and probably other cities, is 3-methylfuran (3MF). It has been speculated that 3MF is derived from the photo-degradation of terpenoid compounds produced by deciduous vegetation. Based on our previous studies showing profound pulmonary toxicities by 3-substituted furan derivatives, we have studied the metabolism and toxicity of 3MF.

Analogous to 4-ipomeanol, 3MF is metabolized *via* cytochrome P450-dependent oxidases to a highly toxic alkylating agent in pulmonary bronchiolar cells. This occurs when 3MF is given ip, or by inhalation at concentrations as low as 20 ppm. We feel these findings indicate that 3MF should be evaluated for potential public health significance, particularly since it does not appear to be derived from an industrial source. It seems possible that 3MF in sufficient concentrations could aggravate bronchial disease in man, since it causes a striking bronchiolar necrosis in experimental animals after inhalation. It also seems reasonable that 3MF should receive attention as a potential environmental carcinogen, since most alkylating agents are carcinogenic. In our continuing studies we are attempting to further characterize the metabolism, and the toxicologic properties of 3MF. We are also examining its mutagenicity as an initial step in evaluating it as a potential pulmonary carcinogen.

5. Biochemical and Morphological Correlates in Drug-Induced Lung Inquiry. (Boyd) Although our previous studies have demonstrated the participation of reactive metabolites in chemically-induced lung injury, the precise mechanisms of chemical/tissue interactions in the pathogenesis of the lesions has not been elucidated. In this project we are approaching these questions using a combination of biochemical measures coupled with

morphologic techniques, including high-resolution light microscopy, electron microscopy, and autoradiography. Through careful study of the time courses and dose-dependencies of the metabolism of the toxins, the site of cellular interactions of reactive metabolites, changes in selected cellular biochemical parameters, and morphologic alterations, we hope to determine the critical chemico-biologic events leading to cell death. Compounds currently under study in this project include 4-ipomeanol, α-naphthylthiourea, nitrofurantoin, 3-methylfuran, paraquat, and bleomycin.

6. Genetic factors in target organ toxicities. (Boyd, Dutcher, Statham) In this project we are exploring the possibility that species and strain differences can be utilized to help define the biochemical bases for the selective destruction of various tissues by certain chemical agents. Our studies thus far clearly illustrate that genetic and constitutional factors may play a fundamental role in the expression and regulation of target organ toxicities by chemical agents that can be activated by metabolism in a variety of tissues. In continuing studies, we are attempting to further elucidate the metabolic parameters that apparently underlie these genetic differences in target organ susceptibilities to chemical toxins and carcingens.

7. Role of cytochrome b_5 in the pulmonary activation and toxicity of 4-ipomeanol. (Boyd, Sasame) Previous studies clearly indicate the obligate participation of cytochrome P450 in the metabolism of 4-ipomeanol (IPO) to a toxic alkylating agent by lung or liver microsomes. The striking specificity for activation of IPO in the lung, vs. the liver, remains a mystery. We have recently obtained intriguing results which suggest an answer. Activation of 4-ipomeanol involves a two-electron transfer during the oxidative transformation. Although P450 can apparently participate in the transfer of both electrons, another factor appears to be rate-limiting for the transfer of the second electron by the pulmonary enzyme system, but not by the liver system. Cytochrome b_5 appears to be an agent through which the transfer of the second electron can be facilitated in the lung microsomal enzyme system. An antibody prepared against highly purified cytochrome b_5 almost completely inhibits the lung microsomal metabolism of 4-ipomeanol, but has little effect on the liver microsomal metabolism of the compound. Further investigations are underway to determine if cytochrome b_5 plays a critical role in the target organ selectivity of 4-ipomeanol toxicity *in vivo*.

8. *In vitro* and *in vivo* studies of stable metabolites of metabolically-activated pulmonary toxins as an approach to the identification of highly reactive, lung-toxic metabolites. (Boyd, Dutcher, Jones, Buckpitt) Highly reactive, toxic drug metabolites combine covalently with tissue macromolecules, rearrange spontaneously, or are further metabolized; thus, in most cases they cannot actually be isolated and identified. One alternative in identifying the chemical nature of these highly reactive species therefore is to carefully determine the characteristics of the secondary metabolites or rearrangement products, in order to permit a rational speculation as to the nature of the intermediates from which they were derived. We are currently applying this reasoning to the study of the

stable metabolites, formed both in *in vivo* and *in vitro* systems, of 4-ipomeanol, nitrofurantoin and 3-methylfuran.

9. Role of reduced glutathione (GSH) as a modulator of drug-induced pulmonary toxicities. (Boyd) Other investigators have shown that liver GSH can combine with highly reactive electrophilic drug metabolites to form non-toxic excretable derivatives, and it can thereby protect the liver from potentially hepatotoxic metabolites. We have recently found that GSH may play a similar "protective" role against the lung lesions induced by an electrophilic metabolite of 4-ipomeanol. These studies are being extended to examine the hypothesis that GSH may play a general protective role against a variety of lung lesions induced by direct alkylating agents as well as alkylating metabolites.

10. Mechanisms of Tolerance Development to Chemical Injury of the Lung. (Boyd, Statham, Sasame) The development of pulmonary tolerance and cross-tolerance to lung-toxic agents is a well-recognized but poorly understood phenomenon. In this project we are using 4-ipomeanol as a model pulmonary toxin to study biochemical and morphological changes accompanying tolerance development. It appears that there are two major features associated with tolerance development to normally lethal doses of 4-ipomeanol, induced by pretreatment of animals with small, nonlethal doses of 4-ipomeanol: a) a loss of cytochrome P450-dependent mixed-function oxidase activity in lung, b) a change in the relative cellular composition of lung to more resistant cell types (ciliated bronchiolar cells in airways, type II pneumocytes in alveoli). It thus appears that tolerance to 4-ipomeanol may result both from a loss of the capacity to activate the compound in the lung, and a cellular adaptation phenomenon (the two events may actually be the same, since the relative cellular composition shifts to cell types possessing little if any MFO activity). Further studies are underway to determine if mechanisms of tolerance development to 4-ipomeanol also apply to tolerance phenomena with other lung-toxic agents.

11. Structural requirements for pulmonary specificity of 4-ipomeanol activation and toxicity; design and synthesis of potential new chemotherapeutic agents for bronchogenic lung cancer. (Boyd, Dutcher) The purpose of this project is to identify, through structure-activity studies, compounds which possess maximal potency and selectivity for metabolism and alkylation of pulmonary Clara cells, using 4-ipomeanol as the prototype agent. Studies will then be undertaken to elucidate metabolic and/or dispositional factors contributing to the specificity of those agents. We anticipate that these studies will contribute significantly to the further elucidation of the chemico-biologic factors determining target organ selectivities of metabolically-activated toxins. But, perhaps more importantly we hope to identify optimal candidates for evaluation as potential new chemotherapeutic tools for treatment bronchogenic lung cancers which retain MFO activity.

12. Evaluation of specific bronchiolar alkylating agents, with 4-ipomeanol as the prototype, as a potential new class of chemotherapeutic agents for treatment of bronchogenic lung cancers. (Boyd, Statham, Jones, Dutcher) Since our studies have shown that pulmonary Clara cells contain

enzymes which activate pulmonary carcinogens, and since most pulmonary cancers are probably induced by metabolically-activated chemical carcinogens, we suspect that at least some pulmonary cancers, especially those originating from Clara cells, may possess the capacity to activate potentially cytotoxic agents such as 4-ipomeanol. We are therefore undertaking a comprehensive investigation to evaluate the potential antitumor activity of 4-ipomeanol, and related derivatives (above), in a variety of experimental tumor systems. Special emphasis will be placed on systems in which the capacity for activation of 4-ipomeanol is demonstratable. We anticipate extensively evaluating the capacity of viable human tumor specimens to activate the candidate agents; perhaps the identification of tumor cell types, or family of cell types, possessing the ability to activate 4-ipomeanol, will implicate a Clara-cell origin for those tumors. If tumor specimens can be obtained in which the capacity to activate 4-ipomeanol can be demonstrated, we shall attempt to develop a xenograph model using the athymic (nude) mouse as the host. It is probable that such a system will be the best experimental system to evaluate *in vivo* the potential antitumor activity of 4-ipomeanol in a susceptible neoplasm. There is no reason that conventional screening procedures would detect 4-ipomeanol as a candidate agent, unless, by chance, the cells used in the system could activate the compound; this seems to be an unlikely possibility. We are predicting that 4-ipomeanol will show activity only in a very specific family of tumors; i.e., those derived from cells capable of activating the compound. It follows of course that a simple biopsy specimen might eventually be sufficient for the study and the *prediction* of susceptibility (i.e., ability to activate the drug) of a given patient's tumor to a metabolically-activated cytotoxic agent such as ipomeanol.

13. *Mechanism of Toxicity of α-naphtylthiourea (ANTU)*. (Boyd, Statham) ANTU is a relatively specific pulmonary toxin, which has been used extensively by pulmonary physiologists and pathologists to study the pathophysiologic changes of acute pulmonary edema. We have recently demonstrated that ANTU toxicity requires metabolic activation of the thiocarbonyl moiety of the compound, *via* a cytochrome-P450 *independent* mixed-function oxidase in lung. We are therefore continuing our investigations into the relationship between the metabolism and the toxicity of this model compound in order to compare it to other compounds we have studied and elucidate the role of metabolism in chemically-induced pulmonary disease in general.

14. *Role of Renal Metabolism in Chemical-induced Renal Necrosis*. (Boyd, Dutcher, Sasame) In the mouse, but in no other species we have examined, 4-ipomeanol produces extensive renal cortical necrosis, in addition to pulmonary bronchiolar necrosis. We have now established that *in situ* activation of 4-ipomeanol to an alkylating agent in the kidney is responsible for its nephrotoxicity in the mouse. Preliminary studies indicate that cytochrome b_5 can uniquely facilitate the activation of 4-ipomeanol in the mouse kidney, as it apparently does in the lung of the mouse and other species. Also, preliminary autoradiographic studies demonstrate a marked cellular specificity for the metabolism and toxicity of 4-ipomeanol in the mouse kidney. We anticipate that, as with 4-ipomeanol in the lung, it may therefore be possible to identify cell

types in the kidney that possess cytochrome P450-dependent enzymes capable of activating 4-ipomeanol.

15. Development of new experimental approaches to the *in vivo* measurement of chemically-induced peroxidative lipid damage. (Boyd, Buckpitt) Indirect studies have implicated a role for peroxidative lipid destruction in the pulmonary toxicities produced by paraquat and by nitrofurantoin. However, thus far, definitive *in vivo* evidence for peroxidative lipid damage by these agents has not been obtainable by conventional procedures. It has therefore not been possible to establish the relationship, if any, between lipid damage, and the appearance of lung lesions. We have therefore undertaken the attempt to develop newer, more sensitive techniques for assay of altered pulmonary lipids. Included in these studies will be lung lipid profiling by GC-MS procedures, and assay of lipid-derived hydro-carbons, such as ethane and pentane, in the expired air. Particular emphasis is being directed to the latter approach, since it is potentially readily adaptable to the study of drug-induced peroxidative lipid damage in man in a clinical setting.

Publications:

Nelson, S.D., Boyd, M.R., and Mitchell, J.R.: Role of Metabolic Activation in Chemical-Induced Tissue Injury. In Jerina, D.J. (Ed.): *Recent Advances in Study of Drug Metabolism*. American Chemical Society, Washington, D.C., 1977, pp. 155-185

Boyd, M.R., and Mitchell, J.R.: The role of metabolic activation in pathogenesis of acute kidney, and lung injury by xenobiotics. In Bridges, J.W. and Chasseaud, L.F. (Eds.): *Progress in Drug Metabolism*, Vol. 3, New York, John Wiley and Sons (in press, 1977).

Mitchell, J.R., and Boyd, M.R.: Dose Thresholds, Host Susceptibility, and Pharmacokinetic Considerations in the Evaluation of Toxicity from Chemically Reactive Metabolites. In Duncan, W.A.M., and Plaa, G.L. (Eds.): *Proceedings of the First International Congress on Toxicology*. Academic Press (in press, 1977).

Boyd, M.R.: Role of Metabolic Activation in the Pathogenesis of Chemically-Induced Pulmonary Disease; Mechanism of Action of the Lung-Toxic Furan, 4-Ipomeanol. *Environmental Health Perspectives*. 16: 127-138, 1976.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)

U.S. DEPARTMENT OF
HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06513-01-CP

PERIOD COVERED

July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Molecular Pharmacology of Antitumor Agents

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Bruce A. Chabner, M.D., Lab. Chief	CPB,DCT,NCI
	Charles E. Myers, M.D., Senior Investigator	CPB,DCT,NCI
	Michael Boyd, M.D., Ph.D., Senior Investigator	CPB,DCT,NCI
OTHER:	Michael Cohen, M.D., Visting Fellow	CPB,DCT,NCI
	Ross Donahower, M.D., Clinical Associate	CPB,DCT,NCI
	Marc Lippman, M.D., Senior Investigator, Medicine Branch	DCT,NCI
	Richard Bender, M.D., Senior Investigator Medicine Branch	DCT,NCI
	David Rozelle, Student Scientist, Bucknell University (Summer 1977)	

COOPERATING UNITS (if any)

LAB/BRANCH

Clinical Pharmacology

SECTION

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS: 3	PROFESSIONAL: 2-1/2	OTHER: 1/2

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

These studies are directed at understanding the quantitative and qualitative interactions of antitumor agents with target sites in tumor cells. Emphasis has been placed on understanding factors which determine response to antifolates, 5-fluoro-uracil, and the pineal hormone, melatonin at the cellular and biochemical level. In vitro uptake, binding to receptor sites, and cytotoxicity of these agents are examined in tissue culture systems as well as in the whole animal.

PHS-6040
(Rev. 10-76)

1. Determination of tightness of binding and release of methotrexate and dihydrofolate reductase in L1210 leukemia cells, in vitro. In this project we are examining the formation of a tightly bound complex of 3H-MTX and dihydrofolate reductase in leukemic cells in short term culture. Studies thus far have shown that MTX forms a tight complex with a high-molecular weight substance which comigrates with dihydrofolate reductase on gel filtration. When drug is allowed to efflux, radioactivity remaining in the cell is all in the tightly bound form. If cold MTX is then added, the dissociation of labelled drug from the tightly bound complex can be followed and is log-linear with time, indicating a first order "off" rate. Other tightly binding inhibitors will readily displace MTX from this binding site, but thus far physiological folates will not do so. The kinetics of dissociation in vitro are being compared with dissociation of MTX from reductase in a cell-free system using homogeneous enzyme.

2. We are initiating studies of the formation of a tight-binding complex of FdUMP with thymidylate synthetase and methylene-tetrahydrofolate in L1210 cells. The primary questions to be addressed are the kinetics of binding of inhibitor to enzyme in conditions of folate depletion, and in the presence of the antifolates, methotrexate or aminopterin.

3. We have initiated studies of the pharmacology of melatonin, the primary secretion of the pineal gland. There is abundant experimental evidence that melatonin is a potent suppressor of pituitary gonadotrophin secretion and estrogen production in the gonads. We are interested in the possibility that melatonin administered exogenously can suppress the growth of estrogen dependent breast tumors in rats (induced by DMBA); if this is found to be the case, then we will undertake the depth studies of the mechanism of inhibition, including hormonal effects, action through a melatonin-specific receptor protein, or action through elevation of cyclic neucleotides.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 06514-01 CP

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Clinical Pharmacology of Antineoplastic Agents

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Bruce A. Chabner, M.D., Chief, Clinical Pharmacology Branch, COP, DCT, NCI
Charles Myers, M.D., Sr. Investigator, CPB, COP, DCT, NCI
Michael Boyd, M.D., Sr. Investigator, CPB, COP, DCT, NCI

Other: Kenneth Hande, M.D., Clinical Associate, CPB, COP, DCT, NCI
James Doroshow, M.D., Clinical Associate, CPB, COP, DCT, NCI
Gershon Locker, M.D., Clinical Associate, CPB, COP, DCT, NCI
Harold Londer, M.D., Clinical Associate, CPB, COP, DCT, NCI
Ross Donehower, M.D., Clinical Associate, CPB, COP, DCT, NCI
Richard Adamson, Ph.D., Chief, Laboratory of Chemical Pharmacology,DTP,DCT
Peter H. Wiernik, M.D., Chief, Clinical Oncology Branch, BCRC, DCT, NCI
Steven Rosenberg, M.D., Chief, Surgery Branch, COP, DCT, NCI

(continued on next page)

COOPERATING UNITS (if any)

Robert Dedrick, Ph.D., Sr. Investigator, BEI Branch, DRS, NIH
Robert Lutz, Ph.D., Sr. Investigator, BEI Branch, DRS, NIH

LAB/BRANCH Clinical Pharmacology

SECTION Office of the Chief

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
8	5	2

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☒ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purposes of this project are to: (1) describe the kinetics of plasma disappearance, urinary excretion, and other relevant pharmacokinetic parameters of antineoplstic agents in man; (2) relate these kinetic parameters to specific clinical problems such as tissue specific toxicities, clinical effectiveness, or influence of renal or hepatic dysfunction on drug disposition; (3) explore the effect of route of administration on drug pharmacokinetics; and (4) determine drug interactions responsible for alteration in patterns of toxicity or therapeutic effect. Drugs currently under study include methotrexate, allopurinol, maytansine, 5-fluorouracil, and cytosine arabinoside.

PHS-6040
(Rev. 10-76)

Other: Eddie Reid, 2nd Year Medical Student, Yale University School of Medicine, CO-STEP Program
Robert Diasio, M.D., Clinical Associate 1975-76, and currently Asst. Professor, Virginia Commonwealth University, Medical College of Virginia, Richmond, Virginia
Ronald Stoller, M.D., Asst. Professor, Brown University School of Medicine, Providence, Rhode Island
Samuel Jacobs, M.D., Asst. Professor, University of Pittsburgh, Pittsburgh, Pennsylvania
Daniel Zaharko, Ph.D., Senior Investigator, LCP, DTP, DCT, NCI
Joan Bull, M.D., Senior Investigator, MB, COP, DCT, NCI

Project Description:

1. Methotrexate

During the past year, we have collaborated with the primary clinical services of the Cancer Institute in designing and monitoring protocols which use high doses of the antifolate, methotrexate.

a) osteogenic sarcoma - Surgery Branch, NCI
b) head and neck cancer - Surgery Branch, NCI
c) histiocytic lymphoma - Medicine Branch, NCI
d) malignant melanoma - Medicine Branch, NCI
e) intrathecal methotrexate - all services of NCI

These studies have resulted in the definition of safe durations of infusion, drug concentration, and leucovorin rescue for these regimens. In particular, in 50-250 mg/kg infusions for 6 hours, a level less than 9×10^{-7} M at 48 hours was not associated with toxicity, while higher levels were invariably associated with serious toxicity unless increased leucovorin was administered. The need for hydration and fur urinary alkalinization was also demonstrated. In head and neck cancer, a regimen employing 1.5 gm/m^2 for 36 hours, followed by leucovorin, was shown to be safe and effective as a pre-operation regimen.

In addition, a comparison of competitive binding and radioimmunoassay techniques for measuring antifolates is presently under way, and contaminants of commercial methotrexate, as well as urinary metabolites, are being examined for their ability to interfere with these assays.

A new project will be initiated shortly to examine the use of methotrexate by peritoneal dialysis for patients with minimal residual ovarian tumor. This project is based on earlier findings that a barrier to methotrexate diffusion into peripheral blood exists. This should allow the exposure of intra-abdominal tumor to high drug concentrations with minimal systemic toxicity. Extensive pharmacokinetic studies are planned in conjunction with this project.

During the past year, several studies dealing with the effects of methotrexate on bone marrow have been completed. These studies have confirmed the requirements for 10^{-8} M drug as a toxic threshold, have demonstrated recruitment of cells to colony formation during drug exposure, and have shown that both drug concentration above 10^{-8} M and duration of exposure up to 72 hours contribute directly to toxicity. Rescue requirements were also studied and it was shown conclusively that leucovorin must be present in equal or greater concentrations than antifolate to effect rescue. Also, it was shown that both purine and pyrimidines (thymidine) were required for rescue of bone marrow colony forming cells.

2. Allopurinol

In order to determine the mechanism of inhibition of urate synthesis by allopurinol, and reasons for urate nephropathy in lymphoma and leukemia we have initiated an investigation of allopurinol pharmacology. Assays for allopurinol and oxipurinol based on enzyme inhibition and competitive binding to xanthine oxidase have been developed and plasma pharmacokinetics have been assessed. These studies indicate weak inhibition of an oxidized species of xanthine oxidase, with no evidence of progressive enzyme inactivation by oxipurinol. A prolonged t1/2 of 13 hours has been determined for oxipurinol.

3. Maytansine

A phase I trial of the new plant alkaloid maytansine has been conducted in conjunction with the Medicine Branch. A maximum dose of 2 mg/m^2 has been achieved. Responses have been seen in patients with ovarian cancer, lymphoma, and acute lymphocytic and myelocytic leukemia. Pharmacokinetic studies have begun using a competitive tubulin binding assay.

4. Cytosine Arabinoside (ara-C)

We have continued to study the pathways by which ara is metabolized in human leukemic cells, hoping to define mechanisms of resistance. These studies have included:

a) Purification and characterization of dCMP kinase, a sulfhydril-dependent enzyme capable of phospholating ara-UMP, ara-CMP, dUMP, FdUMP, and other nucleotides. Its poor affinity for these substrates may lead to its being rate limiting in the activation of these substrates.

b) Competitive inhibition of cytidine deaminase, a major degradative enzyme, by uridine and analogs, including FUdR and 3-deaza-uridine. This finding suggests possible synergism of these compounds with cytidine analogs and these interactions are being evaluated in experimental tumor systems.

c) Prospective study of the influence of cytidine deaminase and deoxycytidine kinase on ara-C response in human leukemia. No relationship of these enzymes to clinical response was seen.

d) Plasma cytidine deaminase activity: source of this enzyme and effect on ara-C metabolism. Evidence was found that this activity increases with increased granulocyte turn-over or hepatic dysfunction. Its relationship to response to ara-C in AML is being investigated in conjunction with the Baltimore Cancer Research Center.

e) Kinetics of inactivation of cytidine deaminase by tetrahydrouridine. A prototype investigation of the kinetics of enzyme inhibition by competitive binding techniques has been completed. These methods allow precise definition of "on" and "off" rates for tight-binding inhibitors.

f) Activation of 5-aza-cytidine by uridine-cytidine kinase from human leukemic cells. Enzyme isolated from the CEM cell line had very weak affinity for 5-aza-cytidine, raising questions as to the possibility that pathways other than direct activation to the nucleotide might contribute to the cytotoxicity of this drug.

5. 5-Fluorocytosine

Extensive characterization of the anti-fungal activity of this agent was undertaken to prove that the toxic end-metabolite 5-FdUMP is produced in fungi.

Publications

1. Stoller, R.G., Coleman, C.N., Hande, K.R., Chang, P.R., and Chabner, B.A.: Biochemical pharmacology of cytidine analogue metabolism in human leukemia cells. Bibliotheca Haematol. 43: 531-533, 1976.

2. Hande, K.R., Stoller, R.G., Drake, J.C., Myers, C.E., Rosenberg, S.A., and Chabner, B.A.: The value of monitoring plasma methotrexate concentrations during high dose infusions. Europ. J. Cancer, in press.

3. Romolo, J.L., Goldberg, N.H., Hande, K.R., and Rosenberg, S.A.: The effect of hydration on plasma methotrexate levels. Cancer Treatment Rep., in press.

4. Tormey, D.C., Simon, R.M., Lippman, M.E., Bull, J.M., and Myers, C.E.: Evaluation of tamoxifen dose in advanced breast cancer: A progress report. Cancer Treatment Rep. 60: 1451-1459, 1976.

5. Kaplan, H., Myers, C.: Inhibition of thymidylate synthetase by aromatic diamidines: Evidence for both rapid, freely reversible and slowly progressive, non-equilibrium inhibition. J. Pharm. & Exper. Therap., in press.

6. Stoller, R.G., Myers, C.E., and Chabner, B.A.: Analysis of cytidine deaminase and tetrahydrouridine interaction by use of ligand techniques. Biochem. Pharm., in press

7. Diasio, R.B., Bennett, J.E., and Myers, C.E.: The mode of action of 5-fluorocytosine. Biochem. Pharm. , in press.

8. Chabner, B.A., Myers, C.E., and Oliverio, V.T.: Pharmacologic basis of antineoplastic chemotherapy. Seminars in Oncology, in press.

9. Pinedo, H.M., Chabner, B.A.: The role of drug concentration, duration of exposure, and endogenous metabolites in determining methotrexate cytotoxicity. Cancer Treatment Rep., in press.

10. Pinedo, H.M., Zaharko, D.S., Bull, J.M., and Chabner, B.A.: The relative contribution of drug concentration and duration of exposure to mouse bone marrow toxicity during continuous methotrexate infusion. Cancer Res. 37: 445-450, 1977.

11. Drake, J.C., Stoller, R.G., and Chabner, B.A.: Characteristics of the enzyme uridine-cytidine kinase isolated from a cultured human cell line. Biochem. Pharm., in press.

12. Pinedo, H.M., Zaharko, D.S., Bull, J.M., and Chabner, B.A.: Evidence for early recruitment of granulocyte precursors during high dose methotrexate infusion in mice. Blood 48: 301-307, 1976.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 03403-12 M

PERIOD COVERED

July 1, 1976 through September 30, 1977

TITLE OF PROJECT (80 characters or less)

CLINICAL TRIALS AND MISCELLANEOUS CLINICAL INVESTIGATIONS

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

P.I.: Robert C. Young, Chief, Medicine Branch, COP, DCT, NCI
Other: Senior Investigators, MB, COP, DCT, NCI: Richard A. Bender, Bruce A. Chabner, Tom Anderson, Richard I. Fisher, Marc Lippman, Joan Bull, Charles Myers
Vincent T. DeVita, Director, DCT, NCI
Stephen Rosenberg, Chief, Surgery Branch, COP, DCT, NCI
Harmar Brereton, Senior Investigator, Radiation Branch, COP, DCT, NCI
Ralph Johnson, Chief, Radiation Oncology, COP, DCT, NCI
Costin Berard, Head, Hematopathology Section, LP, NCI
Chemotherapy Research Nurses: Susan Hubbard, Bonny Johnson, Audrey Barlock, Jane Cassidy
First Year Clinical Associates, Medicine Branch, COP, DCT, NCI

COOPERATING UNITS (if any)

Radiation Oncology Branch, NCI; Clinical Pharmacology Branch, NCI; Surgery Branch, NCI; Immunology Branch, NCI; Laboratory of Clinical Investigation, NIAID

LAB/BRANCH
Medicine Branch

SECTION

INSTITUTE AND LOCATION
Clinical Oncology Program, National Cancer Institute, NIH, Bethesda, Md. 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
25	18.5	6.5

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The Medicine Branch is a major clinical facility of the National Cancer Institute Its activities are divided between clinical therapeutic trials in patients with cancer and clinically related laboratory research. Clinical trials of cancer treatment in the following diseases are currently underway: Breast Cancer, Ovarian Cancer, Hodgkin's Disease, Non-Hodgkin's Lymphomas, Melanoma, Pancreatic Carcinoma, Sarcomas, Chronic Myelogenous Leukemia. In addition Phase I-II Clinical trials are underway, studying the following new experimental agents: Gallium Nitrate, Acronicine, 5-Azacytidine, Cytembena, Maytansine, and Cis-dichlorodiammine platinum. Laboratory research of the branch is summarized under reports entitled Clinical Pharmacology, Kinetics of Cellular Proliferation, Hematology Studies, Immunological Aspects of Cancer, Hormone Dependence of Malignancy.

MAJOR ACCOMPLISHMENTS IN 1976:

Non-Hodgkin's Lymphoma

Established: the curability of advanced diffuse histiocytic lymphoma with combination chemotherapy, the prognostic factors influencing the likelihood of a curative response, the role of peritoneoscopy in the staging and followup of the lymphomas, and the risk of CNS complications in diffuse lymphomas.

Published: sequential staging techniques which accurately access patterns of spread of the disease, a randomized prospective trial comparing the use of total body irradiation to combination chemotherapy in the lymphocytic lymphomas, and long term followup of chemotherapy treatment of advanced non-Hodgkin's lymphoma.

Characterized: the patterns of relapse after complete remission in the non-Hodgkin's lymphomas.

Ovarian Carcinoma

Published and presented initial results of a randomized prospective trial in advanced ovarian cancer showing a new combination to be more effective than the previous single agent therapy of choice in terms of overall response rate, complete remission rate and survival. Published reviews of clinical trials necessary in gynecologic oncology and advances in the staging of ovarian cancer. Initiated a cooperative institution trial of optimal therapy in early stage ovarian cancer.

Hodgkin's Disease

Published the results of a prospective trial of intensive chemotherapy followed by intensive radiotherapy in advanced Hodgkin's disease and established the risks involved in such therapy. Summarized the existing information on the role of the nitrosourea BCNU in the management of advanced Hodgkin's Disease.

Melanoma

Demonstrated the presence of estrogen receptors in human malignant melanoma.

Chronic Myelogenous Leukemia (CML)

Established the failure of splenectomy to improve the duration of the chronic phase of CML and the activity of Dibromomannitol in the treatment of the chronic phase of CML. Defined the frequency of meningeal leukemia in the blastic phase of CML comparing Vincristine-Prednisone with Cytosine arabinoside-6-thioguanine. Demonstrated the clonal evolution of chromosomal abnormalities in CML.

Other major accomplishments in 1976 will be found in the individual project title reports for the Medicine Branch:

Z01-CM-03420-08-M
Z01-CM-06500-04-M
Z01-CM-03404-06-M
Z01-CM-06700-04-M
Z01-CM-06702-02-M
Z01-CM-06119-08-M
Z01-CM-03423-06-M

Publications:

1. Ihde, D.C., Canellos, G.P., Schwartz, J.H., and DeVita, V.T.: Splenectomy in the chronic phase of chronic granulocytic leukemia: Effects in 32 patients. Ann. Intern. Med. 84: 17-21, 1976.

2. Bunn, P.A., Schein, P.S., Banks, P.M., and DeVita, V.T.: Central nervous system complications in patients with diffuse histiocytic and undifferentiated lymphoma: Leukemia revisited. Blood 47: 3-10, 1976.

3. Ihde, D.C., DeVita, V.T., Canellos, G.P., and Young, R.C.: Effect of splenectomy on tolerance to combination chemotherapy in patients with lymphoma. Blood 47: 211-222, 1976.

4. DeVita, V.T. and Fisher, R.I.: Natural history of malignant melanoma as related to therapy. Cancer Treat. Rep. 60: 153-157, 1976.

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7. Whang-Peng, J., Knutsen, T., Lee, E.C., and Leventhal, B.: Acquired XO-XY clones in bone marrow of a patient with paroxysmal nocturnal hemoglobinuria (PNH). Blood 47: 611-619, 1976.

8. Anderson, T., DeVita, V.T., and Young, R.C.: BCNU in the treatment of advanced Hodgkin's disease: Its role in remission induction and maintenance. Cancer Treat. Rep. 60: 761-767, 1976.

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10. Young, R.C. and DeVita, V.T.: Hodgkin's disease: Chemotherapy. In Conn, H.F. (Ed.): Current Therapy. Phila., Pa., 1976, pp. 290-297.

Publications - Cont.:

11. Hubbard, S. and DeVita, V.T.: Medical oncology drug development and the clinic therapy research nurse. Amer. J. Nurs. 76: 560-565, 1976.

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13. DeVita, V.T., Wasserman, T.H., Young, R.C., and Carter, S.K.: Perspectives on research in gynecologic oncology. Treatment protocols. Cancer 38: 509-525, 1976.

14. Lotz, M.J., Chabner, B.A., DeVita, V.T., Johnson, R.E., and Berard, C.W.: Pathological staging of 100 consecutive untreated patients with non- Hodgkin's lymphomas: Extramedullary sites of disease. Cancer 37: 266-270, 1976.

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25. Whang-Peng, J., Broder, S., Lee, E.C., and Young, R.C.: Unusual clonal evolution in a case of chronic myelogenous leukemia. Acta Hematologica 56: 345-554, 1976.

26. Young, R.C.: Proper staging and chemotherapy in the management of epithelial ovarian cancer. Voprosy Oncologii (In Press).

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28. Fisher, R.I., DeVita, V.T., Johnson, B.L., Simon, R., and Young, R.C.: Prognostic factors for advanced diffuse histiocytic lymphoma following treatment with combination chemotherapy. Amer. J. Med. (In Press).

29. Young, R.C. and Anderson, T.: Ovarian and testicular malignancies: Progress in management of tumors derived from the genital ridge. In Staquet, M. (Ed.): Recent Advances in Cancer Treatment. EORTC Monograph, Raven Press (In Press).

30. Young, R.C., Johnson, R.E., Canellos, G.P., Chabner, B.A., Brereton, H.D., Berard, C., and DeVita, V.T.: Advanced lymphocytic lymphoma: Randomized comparisons of chemotherapy and radiotherapy, alone or in combination. Cancer Treat. Rep. (In Press).

31. Chabner, B.A., Johnson, R.E., DeVita, V.T., Canellos, G.P., Hubbard, S., Johnson, S.K., and Young, R.C.: Sequential staging studies in non-Hodgkin's lymphoma. Cancer Treat. Rep. (In Press).

32. DeVita, V.T., Fisher, R.I., and Young, R.C.: The treatment of diffuse histiocytic lymphoma - New opportunities for the future. In Staquet, M. (Ed.): Recent Advances in Cancer Treatment. EORTC Monograph, Raven Press (In Press).

Publications - Cont.:

33. Hande, K.R., Reimer, R.R., and Fisher, R.I.: A comparison of nodal primary versus extranodal primary histiocytic lymphoma. Cancer Treat. Rep. (In Press).

34. Anderson, T., Bender, R.A., Fisher, R.I., DeVita, V.T., Chabner, B.A., Berard, C., Norton, L., and Young, R.C.: Combination chemotherapy in non-Hodgkin's lymphoma. Results of long-term follow up. Cancer Treat. Rep. (In Press).

35. Anderson, T., Bender, R.A., Rosenoff, S.H., Brereton, H.D., Chabner, B.A., DeVita, V.T., Hubbard, S.P., and Young, R.C.: Peritoneoscopy: A technique to evaluate therapeutic efficacy in non-Hodgkin's lymphoma patients. Cancer Treat. Rep. (In Press).

36. Hande, K.R., Witebsky, F.G., Brown, M.S., Schulman, L.B., Anderson, S.E., Levine, A.S., MacLowry, J.D., and Chabner, B.A.: Sepsis with a new species of corynebacterium. Ann. Intern. Med. 85: 423-426, 1976.

37. Anderson, T., Bender, R.A., Fisher, R.I., et al.: Combination chemotherapy in non-Hodgkin's lymphoma: Results of long-term followup. Cancer Treat. Rep. (In Press).

38. Strayer, D.R. and Bender, R.A.: Eosinophilic meningitis complicating Hodgkin's disease: A report of a case and review of the literature. Cancer (In Press).

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 03404-06 M

PERIOD COVERED
July 1, 1976 through September 30, 1977

TITLE OF PROJECT (80 characters or less)

IMMUNOLOGIC ASPECTS OF CANCER

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

P.I.: Richard I. Fisher, Senior Investigator, MB, COP, DCT, NCI

Others: Tom Anderson, Senior Investigator, MB, COP, DCT, NCI
Robert C. Young, M.D., Chief, Medicine Branch, COP, DCT, NCI
Gilbert A. Mandell, Clinical Associate, MB, COP, DCT, NCI

COOPERATING UNITS (if any)

LAB/BRANCH
Medicine Branch

SECTION

INSTITUTE AND LOCATION
Clinical Oncology Program, National Cancer Institute, N.I.H., Bethesda, Md. 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.5	1.5	1.0

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of the project is to study alterations in the immune system caused by malignancy and chemotherapy. The status of the immune system in Hodgkin's and non-Hodgkin's lymphomas is being evaluated before, during, and after chemotherapy. Immunotherapy of nodular non-Hodgkin's lymphomas with MER will be evaluated and a randomized clinical trial with in vitro monitoring of the immune system is beginning. The status of the immune system in patients with ovarian cancer is being investigated. An animal model of ovarian cancer provides opportunities for studies of immunotherapy and chemotherapy. Studies of the immunosuppressive properties of chlorozotocin, a new non-marrow toxic nitrosourea, are underway in both humans and mice. Infection in immunosuppressed host is being evaluated. A retrospective review of 156 cases of Candidiasis detected at post mortem examination is being completed.

Publications:

1. DeVita, V.T., Goodell, B., Hubbard, S., Geelhoed, G.W., and Young, R.C.: Pneumocystis Pneumonia in patients with cancer: Clinical Pneumocystis Pneumonia in patients with cancer: Clinical Setting. Nat'l Cancer Inst. Monogr. 43: 41-46, 1976.

2. Young, R.C. and DeVita, V.T.: The treatment of pneumocystis carinii pneumonia: Current status of the regimens of pentamidine isethionate and pyrimethamine - sulfadiazine. Nat'l Cancer Inst. Monogr. 43: 193-198, 1976.

3. Fisher, R.I., Jaffe, E.S., Braylan, R.C., Andersen, J.C., Tan, H.K.: Immunoblastic lymphadenopathy: Evolution into a malignant lymphoma with plasmacytoid features. Am. J. Med. 61: 553-559, 1976.

4. Fisher, R.I. and Young, R.C.: Immunologic aspects of Hodgkin's Disease. In Waters, H. (Ed.) Handbook of Cancer and Immunology. Garland Publishing New York. (In Press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 03420-08 M

PERIOD COVERED
July 1, 1976 through September 30, 1977

TITLE OF PROJECT (80 characters or less)
Kinetics of Cellular Proliferation

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

P.I.: Robert C. Young, Chief, MB, COP, DCT, NCI

Others: Bruce A. Chabner, Chief, Clinical Pharmacology Branch, COP, DCT, NCI
Charles Myers, Senior Investigator, MB, COP, DCT, NCI
William McGuire, Senior Investigator, CTEP, DCT, NCI
Joan Bull, Senior Investigator, MB, COP, DCT, NCI
Harmar Brereton, Senior Investigator, ROB, COP, DCT, NCI
Stanley Shackney, Senior Investigator, CPB, COP, DCT, NCI
Paul Bunn, Senior Investigator, NCI-VA, COP, DCT, NCI
Oliver Alabaster, Visiting Fellow, MB, COP, DCT, NCI
John Smyth, Visiting Fellow, MB, COP, DCT, NCI
Paul Chretien, Senior Investigator, SB, COP, DCT, NCI

COOPERATING UNITS (if any)
Clinical Pharmacology Branch, NCI
Radiation Branch, NCI
Surgery Branch, NCI

LAB/BRANCH
Medicine Branch

SECTION
Cellular Kinetics Section

INSTITUTE AND LOCATION
Clinical Oncology Program, National Cancer Institute, NIH, Bethesda, MD 20014

TOTAL MANYEARS: 4.0	PROFESSIONAL: 2.0	OTHER: 2.0

CHECK APPROPRIATE BOX(ES)
☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The primary goal of the Cellular Kinetics Section is to develop a better understanding of the kinetic behavior of normal and tumorous tissues and to use such information to design optimal forms of chemotherapy. Toward this end both animal studies and studies in patients have been undertaken. This year alterations in DNA synthesis induced by chemotherapy in normal and tumorous tissues have been studied after 5-FU, Methotrexate and 5-azacytidine. Nucleoside pool size alterations have been defined after 5-FU therapy in vivo. Kinetic studies in patients with ovarian cancer after methotrexate therapy and studies in patients with chronic myelogenous leukemia after 5-azacytidine have been carried out. Studies are presently underway studying alterations in DNA synthesis induced by Methotrexate in head and neck tumors. Flow microfluorometry studies of ascites tumor from patients with breast and ovarian tumors have been sequentially analyzed after treatment. Additional studies will include flow microfluorometry studies in diffuse histiocytic lymphoma as well as studies of intraperitoneal 5-FU studies in patients with ovarian cancer with malignant ascites. Cellular kinetic studies in an ovarian carcinoma murine tumor model are presently underway.

Publications:

1. Young, R.C., Rosenoff, S.H. and Olson, H.M.: Alteration in DNA synthesis in cardiac tissue and light microscopic and ultrastructural cardiac damage induced by Adriamycin *in vivo*. *Adriamycin Review*, Part II, 149-159, 1975.

2. Young, R.C., Goldberg, D., Grotzinger, K.R.: Alterations in ^{3}H-Thymidine incorporation into DNA induced by Methyl CCNU [1-(2-Chloroethyl)-3-(4-Methyl Cyclohexyl)-1-Nitrosoureas] in normal and tumorous tissues *in vivo*. *Cell and Tissue Kinetics.* 9: 325-332, 1976.

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7. Myers, C.E., McGuire, W.P. and Young, R.C.: Adriamycin: Amelioration of Toxicity by Alpha-Tocopherol. *Cancer Treatment Rep.* 60: 961-962, 1976.

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 03423-06 M

PERIOD COVERED
July 1, 1976 through September 30, 1977

TITLE OF PROJECT (80 characters or less)

IN VITRO MARROW GRANULOCYTE CULTURE

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Joan M. Bull, Senior Invest., MB, COP, DCT, NCI
Other: Mark Lippman, Head, MBCS, MB, COP, DCT, NCI
Peter Carmeci, Investigator, Dept. Tech. Dev., NIHLI
Stephan Ladisch, Investigator, PO, COP, DCT, NCI
Frederick Applebaum, Investigator, PO, COP, DCT, NCI
Ronald Atkinson, Physical Science Project Officer, DCT, NCI

COOPERATING UNITS (if any)
Dept. of Technical Development, NIHLI
Pediatric Oncology Branch, COP, DCT, NCI

LAB/BRANCH
Medicine Branch

SECTION

INSTITUTE AND LOCATION
Clinical Oncology Program, National Cancer Institute, NIH, Bethesda, Md. 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
6	3	3

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The laboratory of in vitro marrow culture is concerned with granulopoiesis and granulopoietic regulation. During the past year the in vitro - in vivo effects of hyperthermia to 42°C have been examined in normal human and murine bone marrow and peripheral blood. Clinical hyperthermia effects on peripheral blood CFU-C have been documented. The effect of immunostimulation on in vivo - in vitro granulopoiesis has been investigated. A clinical correlation confirming in vitro observations of the relative marrow sparing effect of the new nitrosourea chlorozotocin in comparison to BCNU was completed. A new photometric scanner has been developed to improve statistical results with human CFU-C. Future projects are designed to study the temporal relationship of the additive toxic effect of hyperthermia and various classes of chemotherapeutic agents on human CFU-C and human tumor cultures; to develop and purify a new CSF from human mammary tumor; and to differentiate whether the stimulation of granulopoiesis by BCG immunostimulation is a direct response of CFU-C or results from an increased macrophage CSA production.

Objectives:

1. Effect of BCG Pretreatment on Myeloid Recovery from Cyclophosphamide Induced Leukopenia.

Patients receiving immunotherapy are reported to have less myeloid suppression with chemotherapy. To examine this observation, we investigated the effect of BCG pretreatment on the granulopoietic recovery of mice given cyclophosphamide. Test animals received intraperitoneal BCG organisms. Control animals were given saline. Eight days later mice in both groups received cyclophosphamide 300 mg/kg. While nadir peripheral WBC occurring at day 3 was similar in both groups, the mean WBC count was significantly higher in the BCG treated animals on days 4, 5, and 6 post-cyclophosphamide. Bone marrow CPU-C elevation was earlier and higher in the BCG treated group. These findings indicate that pretreatment with BCG accelerated recovery from cyclophosphamide induced leukopenia in the mouse.

2. The *In Vitro* Sensitivity of Human and Murine Hemopoietic Precursor Cells to Chlorozotocin (CLZ) and 1-3-Bid(2Chloroethyl)-1-Nitrosourea (BCNU).

The delayed cumulative hematologic toxicity of the chloroethyl nitrosourea antitumor agents has limited their clinical usefulness. CLZ was designed to modify dose limiting myelotoxicity of the nitrosoureas. The sensitivity of human and mouse CFU-C was determined after *in vitro* incubation with CLZ and BCNU. Results indicated that CLZ is significantly less toxic to human and murine bone marrow than BCNU in concentrations observed in clinical use of these agents.

3. Photometric Macrotube Scanning

A new photometric macrotube scanner to assay CFU-C was designed this year. This has improved ability to detect colony formation using a reticulated photometric light scattering effect. This machine is now used to obtain data on early formation of human myeloid colonies.

4. Fractionation of Human Bone Marrow Prior to Cryopreservation for Autologous Reconstitution.

Cryopreserved autologous bone marrow stem cells protect from the effects of otherwise lethal total body irradiation. Mature myeloid cells within whole marrow lyse after freezing causing clumping and injury of stem cells. Using a discontinuous flow blood cell separator to fractionate human marrow the volume of marrow was reduced by 90%. The mature granulocytes that interfere with stem cell preservations were reduced by 50% while only 15% of the CFU-Cs were lost according to pre and post fractionation assays.

5. Abbott Laboratory CSA

Abbott Laboratories have a NCI funded contract to have prepared a quantity of human colony stimulating factor for human use in smoldering leukemia and aplastic anemia. An IND application has been submitted to the FDA. In addition, the in vitro culture laboratory has available an international standard CSA available to outside investigators and is responsible for evaluating the outside results with this standard.

6. Hyperthermic Effect on CFU-C

In vitro hyperthermia from 38-42°C was tested in murine bone marrow, human bone marrow, and human peripheral blood. Heat of 41-42°C to 2 hrs. was found to be stimulatory. Heat of 42°C >2 hrs. was toxic to murine CFU-C but not to human marrow or peripheral CFU-C. In vivo clinical studies during hyperthermia show stimulation of P.B. CFU-C.

Future Course of the Projects:

Coordinating with developing clinical trials, an in vivo - in vitro investigation of the effect and temporal relationship of hyperthermia plus nitrogen mustard, 5-fluorouracil, chlorozotocin, cis-platinum, and the hypoxic cell sensitizer RO-6051 in human CFU-C will continue. In addition, it is planned to develop a parallel in vitro culture system of human tumors utilizing methodology of Salmon, et al.

Several human mammary cancer lines have shown stimulatory activity in in vitro CFU-C. Serum free media from these cells are being produced and the purified substance will be examined for ability to stimulate short and long term in vitro granulopoiesis.

Examining the effect of BCG immunostimulation on granulopoiesis will continue with further studies to differentiated effects of the stimulation on CFU-C or the macrophage population secreting endogenous CSF.

Publications:

1. Bull, J.M. and Chretien, P.: Hyperthermia in cancer therapy. JAMA 235: 223, 1976.

2. Pinedo, H.M., Chabner, B.A., Zaharko, D.S., Bull, J.M.: Evidence for early recruitment of granulocyte precursors during high dose methotrexate infusions in mice. Blood 48: 301-309, 1976.

3. Schein, P.S., Anderson, T., McMenamin, M.G., Bull, J.: Streptozotocin, chlorozotocin, and related nitrosourea antitumor agents. Chemotherapy 7: 159-165, 1976.

4. Pinedo, H.M., Zaharko, D.S., Bull, J.M., Chabner, B.A.: The reversal of methotrexate cytotoxicity to mouse bone marrow cells by leukovorin and nucleosides. Ca. Res. 36: 4418-4424, 1976.

Publications - Cont.:

5. Ingle, J.N., Tormey, D.C., Bull, J.M., and Simon, R.M.: Bone marrow involvement in breast cancer: Effect on response and tolerance to combination chemotherapy. Cancer 38: 1272, 1976.

6. Pinedo, H., Zaharko, D., Bull, J., and Chanber, B.: The relative contribution of drug concentration and duration of exposure to mouse bone marrow toxicity during continuous methotrexate infusion. Ca. Res. 37: 445-450, 1977.

7. Tormey, D.C., Simon, R.M., Falkson, G., Bull, J., Band, P., Perlin, E., and Blom, J.: Evaluation of adriamycin and dibromodulcitol in metastatic breast cancer. Ca. Res. 37: 529-534, 1977.

8. Bull, J.M., Tormey, D.C., Li, S.H., Carbone, P.P., Falkson, G., Blom, J., Perlin, E., and Simon, R.: A randomized comparative trial of adriamycin versus methotrexate in combination drug therapy. Cancer (In Press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 06119-08 M

PERIOD COVERED

July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Cytogenetic and Hematopoietic Stem Cell

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

Principal Investigator: Jacqueline Whang-Peng, Head, Cytogenetic Oncology Sec., MB, COP, DCT, NCI

Other Investigators: Wind-Show Cheng, MB, COP, DCT, NCI
Susan Sieber, Lab. Chem. Pharm., ET, DCT, NCI
Harmar Brereton, ROB, DCBD, NCI
Harvey Gralnick, Clin. Path. Dept., CC
Arthur Nienhauis, H., IR, NIH
Alan Andrews, DCBD, NCI
Paul Chang, BCRD
Ira Green, Lab. Immunology, NIAID
Arnold Ratner, Private Practice
Joan Bull, COP, DCT, NCI
William Banfield, C, LP
Ian Magrath, Pediatric Oncology, COP, DCT, NCI
Fred Li, Epidemiology Br., NCI
Turid Knutsen, MB, COP, DCT, NCI
Elaine Lee, MB, COP, DCT, NCI

COOPERATING UNITS (if any) Laboratory of Immunology, NCI; Pediatric Oncology Branch, COP, DCT, NCI; Laboratory of Chemical Pharmacology, ET, DCT, NCI; Clinical Pathology, ET, DCT, NCI; Radiation Oncology Branch, NCI; Dermatology Branch, NCI; Epidemiology Branch, NCI; BCRC, VA Hospital

LAB/BRANCH

Medicine Branch

SECTION

Cytogenetic Oncology

INSTITUTE AND LOCATION

Clinical Oncology Program, NCI, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The areas of investigation are:

1. Cytogenetic studies of human neoplastic, hematological, and congenital diseases and of members of high-risk cancer families.

2. Observations of chromosomal aberrations and teratogenic effects of mitotic inhibitors, such as, maytansine, azaribine, colchicine, VCR, VP16, VM26, and other Phase I drugs using in vivo and in vitro techniques.

3. Characterization of human stem cells morphologically, histochemically and by electron microscope studies as well as the elucidation of the cellular kinetics and differentiation. We are using stem cells from the peripheral blood of normal subjects and patients with refractory anemias or other myeloproliferative disorders.

4. Detection of sister chromatid exchange in patients with xeroderma pigmentosa and in cancer families.

5. Studies involving hyperthermia in treatment of various malignant diseases.

6. Cytogenetic studies of direct preparations and tissue culture cell lines established from pleural fluids and ascities from patients with Burkitt's lymphoma.

7. Establishing a standardized karyotype of two Guinea pig species, Cavia cobaya and cavia porcellus.

Publications:

1. Whang-Peng, J., Lutzner, M., Edelson, R., and Knutsen, T.: Cytogenetic Studies and Clinical Implications in Patients with Sezary Syndrome. Cancer 38:861-867, 1976 (August).

2. Whang-Peng, J., Lee, E.C., Forni, G., and Green, I.: Mutant Lines of Guinea Pig L_2C Leukemia: II. Comparative Cytogenetic Studies and Banding Analyses of Normal and Leukemic Karyotypes. JNCI 57:897905, 1976.

3. Whang-Peng, J., Knutsen, T., Ziegler, J., and Leventhal, B.: Cytogenetic Studies in Acute Lymphocytic Leukemia. Special Emphasis in Long-Term Survival. Medical and Pediatric Oncology 2:333-351, 1976.

4. Whang-Peng, J., Sonstegard, R., and Dawe, C.: Cytogenetic Study of Malignant Lymphoma in Northern Pike (Esox lucius) from the U.S.A. Cancer Research 36:3554-3560, 1976.

5. Whang-Peng, J., Broder, S., Lee, E., and Young, R.C.: Unusual Clonal Evolution in a Case of Chronic Myelogenous Leukemia. Acta Haematologica 56:345-354, 1976.

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7. Whang-Peng, J.: Banding in Leukemia: Techniques and Implications. JNCI 58:3-8, 1977.

8. Law, I.P., Hollinshead, A.C., Whang-Peng, J., Dean, J.H., Oldham, R.K., Herberman, R.B., and Rhode, M.C.: Familiar Occurrence of Colon and Uterine Carcinoma and of Lymphoproliferative Malignancies. II. Chromosomal and Immunologic Abnormalities. Cancer 39:1229-1235, 1977.

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11. Barr, R.D., Whang-Peng, J., and Perry, S.: Preparation of Mononuclear Cell Subpopulations from Normal Human Peripheral Blood. *Biomedicine* (in press)

12. Barr, R.D., Whang-Peng, J., and Perry, S.: Regulation of Human Haemopoietic Stem Cell Proliferation by Syngeneic Thymus-derived Lymphocytes. *Acta Haematologica* (in press)

13. Chou, Janice Y., Wintraub, R.D., Rosen, S.W., Whang-Peng, J., Sussman, H.D., Haughom, J.R., Robinson, J.C.: Synthesis of alpha subunit of Human Chorionic Gonadotrophin by Presumptive HeLa Cells. *In Vitro* 12:589-594, 1976.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 06500-04 M

PERIOD COVERED
July 1, 1976 through September 30, 1977

TITLE OF PROJECT (80 characters or less)

CLINICAL PHARMACOLOGY OF ANTINEOPLASTIC AGENTS

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

P.I.: Tom Anderson, Senior Investigator, MB, COP, DCT, NCI
Richard Bender, Senior Investigator, MB, COP, DCT, NCI

Other: Richard Fisher, Senior Investigator, MB, COP, DCT, NCI
Charles Myers, Senior Investigator, MB, COP, DCT, NCI
Bruce A. Chabner, Chief, Clinical Pharmacology Branch, COP, DCT, NCI
Leonard Zwelling, Clinical Associate, MB, COP, DCT, NCI
Robert Warren, Clinical Associate, MB, COP, DCT, NCI
Donald Murinson, Research Fellow, MB, COP, DCT, NCI
Richard Adamson, Chief, Laboratory of Chemical Pharmacology, EXT, NCI
Kurt Kohn, Laboratory Molecular Pharmacology, NCI

COOPERATING UNITS (if any)
Laboratory of Chemical Pharmacology, EXT, NCI
Division of Research Services, BEI, NIH

LAB/BRANCH
Medicine Branch

SECTION
Clinical Pharmacology Section

INSTITUTE AND LOCATION
Clinical Oncology Program, National Cancer Institute, N.I.H., Bethesda, Md. 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
6	6	0

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

A variety of projects are underway to develop a greater understanding of the mechanisms of various antitumor agents to improve upon the efficacy of clinical chemotherapeutic trials with these agents. Ongoing work with the antifolate compounds, nitrosoureas, vinca alkaloids, 5-FU, and the platinum coordination compounds has been carried out over the past year and will continue. In vitro studies are being conducted to elucidate the possible role of drugs which expedite methotrexate transport, specifically the vinca alkaloids. The biological properties of nitrosourea derivatives are under study, and a new non-myelosuppressive analogue, chlorozotocin, has entered clinical trials during the past year. Pharmacokinetic evaluation of this agent has been begun. Methods are being developed to assay the various metabolites of 5-fluorouracil, as well as the metabolites of the normal pyrimidine precursors of nucleic acids. The platinum coordination compounds are being investigated to determine the mechanisms of binding to nucleic acids and/or nuclear histone proteins. The nitrosoureas are being evaluated in terms of immunosuppressive characteristics. The work will be carried out in terms of transport of nitrosoureas into the central nervous system and other tissues.

PHS-6040
(Rev. 10-76)

Project Description:

The Section of Clinical Pharmacology of the Medicine Branch is concerned with studying the mechanism of action, disposition and pharmacological correlates of toxicity of established agents, as well as the initial trials of new agents. Areas of specific interest in the past year have included the following:

1. Antifolate Pharmacology and Pharmacokinetics.

Studies aimed at assessing the critical determinants for vincristine (VCR) augmentation of methotrexate (MTX) uptake (i.e. MTX and VCR doses and the interval between their administration) have recently been completed in a L1210 mouse tumor model in vivo. It appears that VCR must be administered simultaneously with MTX to augment in vivo transport. These studies prompted a more detailed analysis of MTX transport at "high-dose" concentrations in human lymphoblast cells which have elucidated, for the first time, a high affinity, low capacity carrier and a low affinity, high capacity carrier for MTX transport. Current studies are elucidating the kinetics of VCR transport in human lymphoblasts. Concurrent animal studies are directed at the mechanisms of VCR resistance in an attempt to pinpoint areas for future investigations in human cells. In addition, the pharmacology of tritiated VCR has recently been completed in human subjects.

2. Nitrosoureas.

The goal of this laboratory has been to pursue the investigation of the structure-activity characteristics of the various nitrosourea antitumor agents. Previous work has demonstrated that molecular manipulation can alter the ability of these compounds to produce permanent diabetes in animal models, depending upon the characteristics of the alkyl side chain. By irradicating the diabetogenecity characteristics of streptozotocin, a new compound chlorozotocin has been developed which appears to have an enhanced therapeutic ratio while maintaining antitumor activity. Further investigations on the effects of chlorozotocin induced lymphocytopenia are underway, as well as possible effects of chlorozotocin inhibition of lymphocyte transformation. Chlorozotocin has entered Phase I trial, and pharmacokinetic study of this compound in man is being undertaken. Current laboratory work in this section is related to further attempts to identify the pharmacologic effects of this compound and its analogues on DNA synthesis, protein synthesis, and lymphocyte function.

3. 5-Fluorouracil.

Work is being carried out by Dr. Donald Murinson in conjunction with Dr. Charles Myers of the Laboratory of Clinical Pharmacology on the mechanism of overcoming resistance of 5-fluorouracil in susceptible tumor and normal tissues. Preliminary work by Dr. Myers suggests this be due to the rapid build up of deoxyuridilate. New assay techniques are being developed to determine whether it is the buildup of this precursor of nucleic acids or some other mechanism which determines the development of resistance to 5-fluorouracil.

4. Platinum Coordination Compounds

In conjunction with Dr. Kurt Kohn of the Laboratory of Molecular Pharmacology work is being carried out utilizing the alkaline elution techniques developed by Dr. Kohn to investigate the interaction of cis- and trans- dichlorodiammine platinum and other analogues with nucleic acids and cellular proteins. Tentative work suggests that while the trans-platinum compounds, which are inactive in terms of antitumor effects, interact with DNA to produce predominantly protease sensitive DNA crosslinking in contrast to the DNA-DNA cross-linking of the cis-platinum compounds. Further work on the characteristics of these interactions are being carried out, as well as looking at some of the platinum analogues.

Publications:

1. Bender, R.A., Bleyer, W.A., Drake, J.C., and Ziegler, J.L. In Vitro correlates of clinical repsonse to methotrexate therapy in drug-resistant patients with acute leukemia and Burkitt's lymphoma. Brit. J. Cancer 34: 484-492, 1976.

2. Warren, E.D., Nichols, A.P., and Bender, R.A. The effect of vincristine on methotrexate uptake and inhibition of DNA synthesis by human lymphoblastoid cells. Cancer Res. (In Press).

3. Bender, R.A., Castle, M.C., Margileth, D.A., and Oliverio, V.T. The pharmacokinetics of [^{3}H] vincristine in man. Clin. Pharmacol. and Therap. (In Press).

4. Warren, R.D. and Bender, R.A. Drug interactions with antineoplastic agents. Cancer Treat. Rep. (In Press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 06700-04 M

PERIOD COVERED

July 1, 1976 through September 30, 1977

TITLE OF PROJECT (80 characters or less)

Clinical Program in Breast Carcinoma

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

Principal Investigator: Marc E. Lippman, Head, MBCS, MB, COP, DCT, NCI

Other Investigators:
Senior Investigators, MB, COP, DCT, NCI
First and Second Year Clinical Associates, MB, COP, DCT, NCI
Chemotherapy Nurses, COP, DCT, NCI
Jane Cassidy, Nurse, MB, COP, DCT, NCI
R. Herberman, Chief, Lab. of Immunodiagnosis, DCBD, NCI
R. Simon, Head, Biostatistical Info. Sys. Unit, COP, DCT, NCI
R. Ruddon, FCRC, Frederick, Maryland
Barbara Curtis, Private Practice

COOPERATING UNITS (if any)

See above

LAB/BRANCH

Medicine Branch

SECTION

Medical Breast Cancer Section

INSTITUTE AND LOCATION

Clinical Oncology Program, National Cancer Institute, Bethesda, Maryland 20014

TOTAL MANYEARS: 3 1/2	PROFESSIONAL: 2 1/2	OTHER: 1

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The Medical Breast Cancer Section is responsible for the development of a clinical and laboratory program directed at breast cancer. Clinical trials of adjuvant chemotherapy and chemoimmunotherapy, and trials in metastatic disease comparing chemotherapeutic, hormonal and chemohormonal regimens are underway. Biochemical and hormonal marker studies are undertaken and coordinated by the Medical Breast Cancer Section.

Project Description:

The Medical Breast Cancer Service was established in July 1972 and the clinical program was initiated in January 1973. It was responsible to the Office of the Associate Director, COP, until its shift to the Medicine Branch in August 1974.

I. Clinical Trials

A. Adjuvant Chemotherapy ± immunotherapy.

1. B135 - This protocol comparing L-PAM to CMF to CMF plus C-parvum was closed due to inadequate accrual. A total of 55 patients entered on study will continue to be followed for DFI, survival and biological marker and immunologic factors.

B. Recurrent disease trials.

1. MB160, a randomized trial of chemotherapy ± hormonal therapy aimed at inducing cell synchrony was initiated in August of 1976. This trial has 20 patients on study and is too early for evaluation.
2. MB151, a randomized trial of hormonal therapy in first recurrent breast cancer in which steroid hormone receptors are to be evaluated prospectively. Only 8 patients have been entered thus far, and this study is too early for evaluation.
3. MB132, a randomized trial of tamoxifen ± halotestin. This trial, nearing completion (68 patients entered), has suggested preliminarily that doses of tamoxifen >12 mg/M^2 are inferior to lower doses, and that tamoxifen plus halotestin is superior in response rate.
4. A Phase II trial of Cis-platinum in breast cancer is underway. Thus far there have been no significant PR's or CR's in this trial.

II. Ancillary Studies

A. Biological Tumor Markers

In cooperation with Dr. Raymond Ruddon, FCRC, we have supplied blood and urine samples from patients for analysis of tumor markers. The results of this program indicate that 45% to 55% of patients pre- and postoperatively, and 93% of patients with metastatic disease, will have elevations of one or more of the following markers: human chorionic gonadotrophin, carcinoembryonic antigen, or N^2, N^2-dimethylguanosine. We are continuing to evaluate new markers such as casein and ferritin, as well as their relationship to tumor burden, site of lesions, pathology, and response to therapy.

B. Steroid Binding Proteins (SBP)

SBP are being prospectively evaluated in all breast cancer samples. These include analyses for androgen, estrogen, glucocorticoid and progestin receptors. In addition, analyses are being performed on melanoma, ovary, colon, male breast and hematologic malignancies.

C. Radionuclide Scanning Procedures

The diagnostic importance of brain, liver-spleen, bone, and gallium scans in breast carcinoma have been evaluated systematically. At the present time the data suggests that brain and liver-spleen scanning are not very useful parameters and gallium scanning appears to be a good screening modality only for the detection of mediastinal lesions. Bone scanning is superior to roentgenographic procedures. Recommendations for the use of these procedures have been formulated.

D. Evaluation of Steroid Binding Proteins

Dr. Brad Thompson was receiving tissue from the Surgery Branch and the Medical Breast Cancer Section for the analysis of steroid binding receptors. This project was transferred to Dr. Marc Lippman in July 1974. A description of the programs can be found in the laboratory report of the Medical Breast Cancer Section.

III. Extramural Activities

A. Breast Cancer Task Force.

Dr. Lippman is on the Steering Committee of the Breast Cancer Task Force.

B. National Surgical Adjuvant Breast Project

Dr. Lippman is on the Endocrine Committee of the National Surgical Adjuvant Breast Project.

Publications:

1. Tormey, D.C., Falkson, G., Perlin, E., Bull, J., Blom, J. and Lippman, M.E.: Evaluation of an Intermittent Schedule of Dibromodulcitol in Breast Cancer. *Cancer Treatment Reports* 60:1953-1596, 1976.

2. Tormey, D.C., Simon, R.M., Lippman, M.E., Bull, J.M. and Myers, C.E.: Evaluation of Tamoxifen Dose in Advanced Breast Cancer - A progress report. *Cancer Treatment Reports* 60:1451-1460, 1976.

3. Young, R.C., Lippman, M.E., DeVita, V.T., Tormey, D.C. and Bull, J.: Perspectives in the Treatment of Breast Cancer. 1977 *Annals of Internal Medicine* 86: 784-798, 1977.

4. Morgan, L.R., Schein, P.S., Wooley, P.V., Hoth, D., McDonald, J., Lippman, M.E., Posey, L.E. and Beazley, R.W.: Therapeutic Use of Tamoxifen in Advanced Breast Cancer: A correlation with biochemical parameters. Cancer Treatment Reports 60:1437-1444, 1976.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 06702-02 M

PERIOD COVERED
July 1, 1976 through September 30, 1977

TITLE OF PROJECT (80 characters or less)

Mechanisms of Hormone Dependence of Human Malignancy

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Marc Lippman, Sr. Investigator, MB, COP, DCT, NCI
C. Kent Osborne, Clin. Assoc., MB, COP, DCT, NCI
Marie E. Monaco, NIH Postdoc. Fellow, MB, COP, DCT, NCI
Fred Hendler, Cancer Expert, MB, COP, DCT, NCI
Others: Susan Aitken, Georgetown Univ. Med. School
Karen Huff, Lab Tech, MB, NCI
E. Brad Thompson, Lab. Biochem. NCI
Ronald Kahn, Diabetes Branch, NIAMDD
Richard Knazek, Lab. Pathophysiol., NCI
Lynn Loriaux, Endocrinology Br., NICHD
Linda Green, Howard Medical School
Ronald Barr, OADP, NCI
Geraldine Konior, Res Assoc Johns Hopkins (Deceased)

COOPERATING UNITS (if any) National Institute of Arthritis, Metabolism & Digestive Diseases, Surgery Branch, Lab. Biochem., Diabetes Br., Lab. Pathophysiol., Endocrinology Br., Georgetown Univ. Medical School, Howard Medical School, Pediatric Oncology Br., Pediatric Oncology Johns Hopkins Hospital

LAB/BRANCH
Medicine Branch

SECTION
Medical Breast Cancer Section

INSTITUTE AND LOCATION
Clinical Oncology Program, National Cancer Institute, Bethesda, Maryland 20014

TOTAL MANYEARS: 10	PROFESSIONAL: 10	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

We are investigating the mechanisms whereby steroid and polypeptide hormones stimulate growth and specific protein synthesis in human breast cancer both in tissue culture model systems we have established and in clinical settings.

A. We are studying the prevalency and clinical correlates of specific steroid receptors for estrogen, androgen, glucocorticoid and progesterone in human breast cancer, lymphomatous diseases, melanoma, colon carcinoma, ovarian cancer and male breast cancer.

B. Assays for specific gene products (α-lactalbumin, CEA, casein, fatty acid synthetase, thymidine kinase) have been developed and we are studying the effects of steroid hormones, insulin and prolactin on the activities and synthesis of these proteins.

C. We are investigating glucocorticoid receptors in various subpopulations of normal and leukemic lymphoid cells, including their regulation by mitogens such as phytohemaglutinin. In addition we are studying glucocorticoid receptors in Burkitts Lymphoma, hairy cell leukemia, ANLL and lymphomas.

D. We have developed a prolactin responsive rat mammary cancer cell line in tissue culture. This is the first in vitro long term prolactin dependent system to be characterized and as such, appears particularly promising for the study of the mechanism of prolactin action.

E. We are characterizing an activity derived from bovine mammary gland which appears to be inhibitory of but not lethal to breast epithelial cells in culture and as such may qualify as a putative chalone.

Publications

1. Lippman, M.E. and Huff, K.: A Demonstration of Androgen and Estrogen Receptors in Human Breast Cancer Using a New Protamine Sulfate Assay. Cancer 38:868-874, 1976.

2. Lippman, M.E., Bolan, G. and Huff, K.: The Effects of Estrogens and Antiestrogens on Hormone Responsive Human Breast Cancer in Long Term Tissue Culture. Cancer Research 36:4595-4601, 1976.

3. Lippman, M.E., Bolan, G. and Huff, K.: The Effects of Glucocorticoids and Progesterone on Hormone Responsive Human Breast Cancer in Long Term Tissue Culture. Cancer Research 36:4595-4601, 1976.

4. Lippman, M.E., Bolan, G. and Huff, K.: The Effects of Androgen and Anti-androgens on Hormone Responsive Human Breast Cancer in Long Term Tissue Culture. Cancer Research 36:4610-4618, 1976.

5. Lippman, M.E., Bolan, G. and Huff, K.: Hormone Responsive Human Breast Cancer in Long Term Tissue Culture in Breast Cancer: Trends in research and treatment, ed. by J.C. Heuson, W.H. Mattheiem and M. Rozenzqeig, Raven Press, 111-139, 1976.

6. Thompson, E.B., Aviv, D., and Lippman, M.E.: Variants of HTC Cells with Low Tyrosine Aminotransferinase Inducibility and Apparently Normal Glucocorticoid Receptors. Endocrinology 100:406-419, 1977.

7. Aitken, S. and Lippman, M.E.: A Simple Computer Program for Quantification and Scatchard Analysis of Steroid Receptor Proteins. J. Steroid Biochemistry 8:77-94, 1977.

8. Lippman, M.E., Osborne, C.K., Knazek, R. and Young, N.: In Vitro Model Systems for the Study of Hormone Dependent Human Breast Cancer. Beth Israel Seminars in Medicine. New England Journal of Medicine 296:154-161, 1977.

9. Lippman, M.E., Huff, K. and Bolan, G.: Progesterone and Glucocorticoid Interactions with Receptors in Breast Cancer Cells in Long Term Cultures. Proceedings of the New York Academy of Sciences 286:101-115, 1977.

10. Lippman, M.E., Bolan, G., Monaco, M.E., Pinkus, L. and Young, N.: Model Systems for the Study of Estrogen Action in Tissue Culture in the 3rd International Symposium of the Journal of Steroid Biochemistry: Recent Advances in Steroid Biochemistry 1:1045-1052, 1976.

11. Lippman, M.E., Bolan, G. and Huff, K.: Interactions of Antiestrogens with Human Breast Cancer in Long Term Tissue Culture. Cancer Treatment Reports 60:1421-1430, 1976.

12. Menon, M., Tananis, C.E., McLoughlin, M.G., Lippman, M.E. and Walsh, P.C.: The Measurement of Androgen Receptors in Human Prostatic Tissue Utilizing Sucrose Density Centrifugation and A Protamine Precipitation Assay. J Urol (in press).

13. Konior, G. S., Lippman, M.E., Johnson, G.D. and Leventhal, B.G.: Glucocorticoid Receptors in Subcategories of Childhood Acute Lymphocytic Leukemia. Cancer Research (in press).

14. Fisher, R.I., Neifeld, J.P. and Lippman, M.E.: Estrogen Receptors in Human Malignant Melanoma. Lancet 2(7981):337-340, 1976.

15. Neifeld, J.P., Lippman, M.E. and Tormey, D.C.: Steroid Hormone Receptors in Normal Human Lymphocytes: Induction of glucocorticoid receptor activity by PHA stimulation. J Biol Chem 252:2972-2977, 1977.

16. Monaco, M.E., Lippman, M.E., Bronzert, D. and Tormey, D.C.: Casein Production by Human Breast Cancer. Cancer Research 37:749-754, 1977.

17. Osborne, C.K., Bolan, G., Monaco, M.E. and Lippman, M.E.: Hormone Responsive Human Breast Cancer in Long Term Tissue Culture. IV. Effects of insulin. Proc Nat Acad Sci, USA 73:4536-4540, 1976.

18. Knazek, R., Lippman, M.E. and Chopra, H.: Formation of Solid Human Mammary Carcinoma in vitro. JNCI 58:419-422, 1977.

19. Lippman, M.E., Monaco, M.E. and Bolan, G.: Effects of Estrone, Estradiol, and Estriol on Hormone-responsive Human Breast Cancer in Long-Term Tissue Culture. Cancer Research 37:1901-1907, 1977.

ANNUAL REPORT OF THE NCI-VA MEDICAL ONCOLOGY BRANCH OF THE DIVISION OF CANCER TREATMENT, NATIONAL CANCER INSTITUTE AND THE WASHINGTON VETERANS ADMINISTRATION HOSPITAL

1.0 SUMMARY

1.1 DESCRIPTION

The NCI-VA Medical Oncology Program is an intramural branch of the DCT/NCI/NIH and a section of the Research and Medical Services Washington, D.C. Veterans Administration Hospital (WVAH). The primary goals of the branch are: cClinical therapeutic trials, investigation of patients with malignant disease and laboratory investigations into tumor cell biology. Secondary aims are to provide a service function to the WVAH for the diagnosis and treatment of patients with malignant disease.

1.2 ADMINISTRATION

The branch is a cooperative program between the COP/DCT/NCI/NIH and the VA with a contract signed between the Director DCT and the VA central office (VACO) providing for the transfer of funds from the DCT to VACO and the provision of ward, office, outpatient clinic, and research laboratory space and positions by the VA to the NCI. Both Veteran and non-Veteran patients are entered into clinical protocols approved by the clinical review committee of the WVAH and the NCI. Currently a letter of agreement is being prepared to define the guidelines under which the unit operates in the WVAH. The definition of these guidelines is imperative for the continued research functions of the branch.

1.3 CLINICAL RESOURCES

All clinical and ward supporting work for protocol and non-protocol patients is performed by: 4 senior staff oncologists, 1 infectious disease specialist, 2 pathologists, 8 clinical associates, 2 rotating interns, 1 pharmacist, 2 research nurses, 22 full time and 4 part time nurses, 3 medical technologists, 4 ward and outpatient clinic secretaries, 1 Medical DMT, dietician, 2 food service workers, social worker, a housekeeping aid and a supply technician. The inpatient unit has 30 beds, the outpatient clinic runs 3 days a week in the WVAH outpatient area and 2 days a week on the inpatient ward. This year a satellite ambulatory care program for protocol patients with 5 rooms in the United Inn, Bethesda, Md. has been established through a direct NCI contract.

1.4 CLINICAL PATIENT LOAD

The program assumes complete primary care in and outpatient responsibility for all patient on its protocols (75%) of patient and in addition, primary care for veteran patients deemed likely to have significant benefit by complex treatment regimens by the Chief Oncology (25% of patients; 250-300 total patients in the program). The program provides outpatient care for all WVAH veterans requiring non-protocol chemotherapy (approximately 50-100 patients in FY77).

The program sees 30-40 new patients a month in consultation following referral from the general hospital. The inpatient beds have been over 90% occupied, and there are approximately 360 outpatient visits per month. Non-veteran inpatient occupancy has been 30% of total, and outpatient visits 17% of the total. A sizable number of the veteran patients come for protocol treatment outside the WVAH area. All transportation costs for protocol patients are met by the NCI transferred funds.

The senior staff provides 10 months a year total of inpatient attending on the general medical service, 2 months a year attending on the infectious disease consultation service, supervises the WVAH tumor registry, participates in the WVAH cancer committee and weekly Medical Surgical Chest Conference, and provides multiple conferences for the WVAH through the general hospital education program.

1.5 ONCOLOGY FELLOWSHIP

The NCI-VA MOB provides for clinical training in the subspecialty of Medical Oncology for Clinical Associates primarily assigned to it and to Clinical Associates rotating from the COP/DCT/NCI. In addition, it provides general medical and oncology training to twelve interns from the Medical Service, WVAH that rotate through the inpatient wards.

1.6 LABORATORY RESEARCH FACILITIES AND STAFFING

The laboratory research program in tumor cell biology is carried on in 2500 square feet of wet lab space, and the program has the use of common research space including instrument, storage and animal rooms totaling 1262 square feet. There are 6 senior investigators, 6 post doctoral fellows (PhD), 4 Clinical Associates, and 11 technicians working in this area.

1.7 MAJOR PROBLEM AREAS

The major problem areas developing in FY 77 are: defining the administrative relationship of the program to the VA; the necessity of the unit to provide for all its inpatient and outpatient supportive services and thus the need for backup of these services; the coming and projected leaving of the WVAH Radiotherapist, Dr. Bilek and the lack of adequate radiotherapy facilities for protocol work; the lack of adequate diagnostic radiology capability of the WVAH; the need for easy access to materials for pathology review; the lack of adequate consultative support in certain other WVAH services; the lack of adequate storage space to support the laboratory and clinical research projects; the need for basic support capability such as typing, secretarial, phone lines, an administrative liason with WVAH. These problems are all being dealt with in the developing letter of agreement to which considerable time has been devoted.

1.8 SUMMARY OF CLINICAL INVESTIGATIONS

The major treatment studies of the NCI-VA Medical Oncology Branch involve small cell bronchogenic carcinoma (SCBC). We are aiming for long duration, disease free survival and potential cure. Our current work has established several important principles in the treatment of SCBC. We have shown that intensive induction chemotherapy without modification for myelosuppression increases the complete response rate, and that patients with SCBC can tolerate intense induction on an open hospital ward without the need for prophylactic non-absorbable antibiotics. We have found that the maximum complete response rate to a given drug combination occurs in the first 6 weeks and that by adding sequentially another non-cross reactive drug combination the complete response rate can be doubled. (CMC-VAP Protocol 75-4)

We have tested in a randomized trial the usefullness of a third such combination in sequence and found it has no beneficial effect. (CMC-VAP-VI regimen protocol 75-4). Our current trial yields a complete response rate of 52% for all patients (70% for disease limited to the thorax and 40% for extrathoracic, extensive disease.) This represents a unique chemotherapy only trial in the USA for limited disease patients with SCBC. In addition, several of the first patients treated are disease free 20-24 months after initiation of treatment indicating the possibility for long term survival in this disease. We have found however, that the most important and durable complete responses are those that can be obtained rapidly in the first 6 weeks. Patients obtaining complete responses after this period frequently relapse.

We have determined also the sites of response and relapse in SCBC and have found that lymph nodes, bone marrow respond well while pulmonary disease response less well, and liver and bone respond least well. Relapses were found to occur in sites of prior bulky disease and have led us to test the addition of radiotherapy to these sites. (Protocol 77-1) CNS sancturary disease responds well to high dose nitrosurea therapy with less than 10% of patients at risk developing CNS metastases. Future studies will combine high dose nitrosourea and prophylactic cranial irradiation.

In these studies limited disease patients achieving a rapid complete remission do extremely well, and indicate that these patients when given optimum chemotherapy would benefit from additional therapy directed at sites of bulky disease. In contrast, despite initial good responses patient with extrathoracic disease (particularly disease involving bone and liver have a much shorter median survival and rarely obtain a long term (2 years of more) disease free state. New protocols (77-1) are thus being constructed to add radiotherapy to sites of bulky disease in patients with limited stage SCBC, and for those patients entering a complete response at appropriate times the addition of prophylactic therapy to sancturary sites. For patients with extrathoracic (extensive) disease we are trying to design new induction regimens. The first uses intensive chemotherapy with cyclophosphamide, adriamycin, VP-16, and vincristine (CAPO Regimen) to try to rapidly achieve a complete response followed by CMC-VAP maintenance. (Protocol 77-2)

Because of the need for intensive therapy either at induction or at other times during the course of SCBC it is very important to find new active non-myelosuppressive agents. We are testing three including streptozoticin, high dose methotrexate with leucovorum factor rescue and cis-platium. (Protocols 76-5,76-9).

Because SCBC is highly responsive to radiotherapy, but biologically represents a disseminated disease, we are piloting studies of radiotherapy used as a systemic agent by given it in the hemibody mode. (Protocol 77-3)

We have discovered by careful histologic review, that many patients that previously would have been diagnosed as non-small cell lung cancer, and conversely patients thought only to have small cell lung cancer actually have a mixture of the two lung cancer types. (Mixed histology lung cancer) we have been treating such patients with intensive induction chemotherapy in a fashion similar to that for treating SCBC. We have found these patients respond well but for a short duration. These studies are important in identifying other patients with lung cancer that might benefit from chemotherapy and stress the need for careful histologic review of all lung cancer cases by an experienced lung cancer pathologist.

A small subset of patients with long term (greater than 2 year) disease free survival is being developed by the NCI-VA MOB. In addition, a registry for such patients around the world is also being developed in collaboration with the extramural program.

The major problem in non-small cell lung cancer is to find active chemotherapy regimens. We have been testing high dose methotrexate with citrovorum factor rescue by two dose schedules: (Protocol 76-9) (6 hour 10-2 M MTX and a 30 hour 10-5 M schedule). We have found we can give both regimens safely to patients with lung cancer; however, we have seen no objective responses. Because of the potential use of this form of treatment in adjuvant settings we are completing this trial which we feel will unequivocally show that high dose methotrexate is of no unique value in non-small cell lung cancer. Similarly in another phase II trial we have found Ifosphamide to have little or no activity in nonsmall cell cancer. (Protocol 76-10).

We have found a new nonmyelotoxic combination chemotherapy regimen with bleomycin, methotrexate and streptozoticin (BMS) (Protocol 75-1) that has activity against epidermoid lung cancer. (40% response rate). However, we observed a significant debilitation syndrome related to this BMS chemotherapy and no improvement in survival in responders. We are thus designing a new study.

In gastric carcinoma, our main protocol (76-8) evaluates the effect of sequential administration of two, active, non-cross resistant drug combinations in patients with measurable unresectable disease: 5-flurouracil,Methyl-CCNU, alternating with adriamycin, mitomycin FM-AM. While we have seen only a few responses with this regimen, we have several long term survivors in young patients or patients with surgically debulked disease indicating the way for our future trials.

Phase II trials in gastric carcinoma are evaluating ICRF 159 (Protocol 76-17) an agent reported to be active in other gastrointestinal malignancies.

In prostatic cancer patients we have seen objective responses to single agent chemotherapy with either 5FU or CCNU. Our current major protocol (76-6) evaluates combination chemotherapy with cyclophosphamide and adriamycin in hormone-resistant Stage D prostatic cancer. Objective responses with clinical benefits have been seen in 30% of patients . We are also investigating secondary hormonal manipulation in patients failing chemotherapy, the value of CAT scanning of tumor and serial followup of tumor markers (such as CEA) in these patients. These studies demonstrate the potential benefit of chemotherapy to prostatic cancer patients.

Patients with cutaneous T cell lymphomas including mycosis fungoides and the Sezary syndrome are being staged and then treated with combined modality chemotherapy and electron beam (EB) radiotherapy. (Protocol 76-12). The chemotherapy, in contrast to published studies, utilizes low dose continuous combinations rather than high dose pulses. The drugs used are vincristine, adriamycin, bleomycin, alternating with cyclophosphamide, methotrexate, prednisone. Early stage patients are receiving EB therapy and randomized to receive low dose NH2. Besides evaluation of routine staging procedures, in collaboration with other groups we are studying malignant T cells: by electron microscopy of skin, blood, and lymph nodes; for their role as helper cells ; malignant cell cytogenetics; quantitation of peripheral blood T cell levels; the presence of steroid receptors. Patients are studied for staphylococcal antibody titers, and the use of antithymocyte globulin in a phase II trial. (Protocol 77-4).

Patients with unresectable hepatocellular carcinoma are being treated with a combination of adriamycin and streptozotocin which can be given in full doses in the combination. (Protocol 76-11) Patients are being followed during treatment with alpha fetoprotein levels. A few patients on this and prior studies have done extremely well following chemotherapy with long term responses or disease stability.

We are actively pursing clinical histopathologic correlations of the tumor types studied in the treatment protocols. A large number of patients with SCBC have been subtyped into three classes: lymphocyte-like, intermediate, and a mixed lymphocyte-intermediate group. There appears to be differences in complete response rates in certain groups indicating that subtyping may be important.

A large number of autopsies of patients with SCBC are being reviewed. The studies show that combined modality therapy may be more effective in debulking tumors and we are evaluating the effect of intensive versus moderate dose chemotherapy to aid in design new regimens.

A major histopathologic development has been the discovery of non-small cell lung cancer histologies and cytologies in patients diagnosed initially as having pure SCBC. At present approximately 10% of patients either during life or at necrospey are found to have frankly invasive epidermoid carcinoma. Differentiation of SCBC into large cell types has also been noted.

We are evaluating sequential gastric cytologies and biopsies obtained endoscopically during the course of chemotherapy. High CEA levels have been found in gastric washings. Surgical specimens from patients undergoing gastric resection with the VASAG are being reviewed and correlated with the subsequent clinical course.

Analysis of autopsy specimens in patients dying 1 month after surgical resection of lung cancer continues to reveal interesting information. The different non-small cell tumor types exhibit different biologic behavior with adenocarcinomas metastasizing widely, large cell carcinomas remaining local and epidermoid carcinomas with pleural involvment metastasizing widely. All of these findings occur irrespective of the primary tumor size.

Staging procedures are actively investigated including fiberoptic bronchoscopy (FOB), gastroscopy, peritoneoscopy, and evaluation of tumor markers with therapy. We find FOB to be extremely valuable in obtaining visual and histopathologic evidence of objective disease response and progression, particularly in SCBC. This can be the only such evidence at times. The washings also provide material for cell biologic and cell kinetic studies. Peritonesocopy is being evaluated in SCBC, T cell lymphomas, gastric and hepatic carinomas.

Tumor cell markers which can be used to evaluate disease state with a particular emphasis on determining complete remissions and relapse from such are studied including: calcitonin in SCBC; CEA in gastric cancer, and prostatic cancer.

Patients with solid tumors undergoing intensive induction chemotherapy develop in addition to marked granulocytopenia, fever that is often of unknown etiology. We are evaluating this problem and developing clinical guidelines for these patients. Specific infectious disease problems in solid tumor patients which we are studying include: gram positive coccal bacteremia, gram negative bacillus bacteremia, Pneumocystis carinii pneumonitis, and salmonella infection. In addition, we have had several cases of interstitial lung disease develop in solid tumor patients. We are developing clinical guidelines for managing this complication.

We are conducting a randomized trial of thymosin (Protocol 76-4) in patients with SCBC receiving intensive induction chemotherapy. This is a unique test of thymosin in this setting. We have found no effect on tumor response but correction of *in vitro* abnormalities have been noted.

We are developing a computer program clinical management system to aid in protocol studies. This is done via a terminal linkup with the NIH computer facilitiies. The major goal is for simple continuous update in flow sheet form for clinicians carrying for the patients.

SUMMARY OF LABORATORY STUDIES

In the laboratory, we are asking fundamental questions about tumor cell biology and developing new methods of treatment of solid tumors. Toward this end, we are developing clonal cell lines from human tumors *in vitro* and in immunosuppressed nude mice. These tumors are studied for their growth factor requirements and ability to differentiate.

We are particulrly interested in the growth factor requirements of SCBC. While the tumor replicates well in the patient, when placed in culture in rich medium in most cases, it fails to grow and cells lyse. We are trying to identify the growth factor(s) the patient supplies in order to potentially remove these in developing a new form of therapy. We have found that certain conditioned media from a variety of cells including normal lung supporting cells, produce factors which are required for SCBC to replicate *in vitro*. We have established two lines of SCBC which exhibit some differentiated functions of neural crest cells including: histaminase, neurosecretory granules, and catecholamine synthesis and storage. We are studying the cell kinetics and effect of various differentiating agents on these cells. In addition, the cell lines can be used for chemo and immunotherapy studies.

Other human tumor cell lines have been established and are being characterized including lines from: mycosis fungoides, gastric carcinoma, mesothelioma, and pulmonary large cell carcinoma.

A nude mouse colony has been established but major problems with the quality of mice still exist. A large number of human tumor samples have been transplanted but only a few lines developed because of the general condition of the mice. The tumors of interest that have been serially passaged include: large cell lung cancer, melanomas, gastric, and colon adenocarcinomas, small cell carcinoma, mycosis fungoides, mesothelioma and sweat gland carcinoma.

Hybrid cells are being used to search for human genes coding for malignancy. Human tumor cells are fused to normal diploid rodent cells and the hybrids tested for their ability to form tumors in nude mice, and colonies in soft agar. Of interest, we are taking malignant human tumors growing in nude mice and selecting for hybrids formed *in vivo*.

An alternative approach involves using hybrid cells to test for genes capable of suppressing malignant behavior. In a model Chinese hamster x normal mouse hybrid cell system we have found we can eliminate all mouse chromosomes except #11 as being able in a single copy to exert trans species supression of malignancy.

Using Chinese hamster x mouse hybrids segregating mouse chromosomes we have succeded in mapping mouse genes required for murine leukemia virus.(MuLV). These include genes for two classes of MuLV. Mouse cells are permissive while Chinese hamster cells are non-permissive for both ecotropic and amphotropic MuLV replication. We tested hybrids segregating mouse chromosomes and found that mouse ecotropic replication requires gene(s) on mouse chromosome #5 (Rec-1) while amphotropic replication requires gene(s) on mouse chromosome #8 (Ram-1) which are necessary and may be sufficient for viral replication. In studies of binding of virus evelope glycoprotein (gp71) to parent and hybrid cells we found that mouse chromosome #5 carries gene(s) responsible for these viral receptors and thus might represent the same gene(s) as Rec-1.

We have studied the spontaneous expression of endogenous MuLV in Chinese hamster x mouse hybrid cells segregating mouse chromosomes. We are testing for genes required for viral activiation or suppression. We have found strain differences between AKR, BALB, and NZB suggesting different genetic mechanisms may be operating. We have also found that these processes occur independently of many of the genes mapped previously by mouse sexual genetics. Hybrid cells segregating chromosomes also become more permissive for viral replication and we are testing our human leukemic x rodent hybrids to see if endogenous human virus is released.

A major effort has gone into to mapping human genes required for primate tropic type C virus replication in hybrid cells. Human x mouse and human x Chinese hamster hybrid cells are infected with baboon M7 virus, Wooly monkey SSV virus, feline leukemia virus, endogenous cat CCC virus and then the hybrids are tested for their chromosome and isozyme composition, and ability to support viral replication by DNA polymerase assay and fluorsecent antibody tests. In addition, Dr. Lemons, until recently a member of our lab, in collaboration with Dr. Sher tested hybrids with radioimmunoassays. Dr. Lemons experiments suggested that a gene on human chromosome 6 was required for baboon M7 virus replication and maintenance, possibly in an integrated state in the hybrid cells. Dr. Brown, in independent experiments, has so far failed to confirm this finding and is postulating a different human chromosome in another set of hybrids. In any event, these experiments indicate that hybrid cells can be used for mapping human genes required for viral functions. Similar work is going on with a more complicated SSV system.

In analgous experiments, Dr. Gazdar, Edgell and Oie have been infecting clonal human cells with these viruses, recloning the cells and then fusing these preinfected human cells to rodent cells and testing the resultant hybrids for virus markers and human chromosomes. They have found the mouse genome suppresses production of all 3 viruses and in some cases the M7 virogene is not completely eliminated as human chromosomes are lost. We have developed a co-cultivation technique that allows us to transfer M7 virus from human to mouse cells and should serve as one model of transpecies transfer of xenotropic viruses.

We have used human x rodent hybrids to map several human isozymes. We have shown that peptidase S is assigned to human chromosome 4 while peptidase D is assigned to human chromosome 19. We are also studying the expression of human lung cancer functions including surfactant and multilamellar body production in hybrid cells.

A major effort has gone into comparative gene mapping. We use hybrid cells to map mouse, Chinese hamster and rabbit genes and then compare these with each other and with man. We are determining which linkage groups are conserved in evolution and deriving a map of chromosome evolution. We have found 4 genes on the short arm of human chromosome 1 are conserved on mouse chromosome 4 (PGD, ENO, PGM-2, and AK-2), two genes on human chromosome 15 are conserved on mouse chromosome 9 (MPI, PK3), two genes on human chromosome 19 are conserved on mouse chromosome 7 (GPI, PEPD), and two genes on human chromosome 4 are conserved on mouse chromosome 5 (PEPS, PGM-1). In addition to the conserved linkages a variety of other new mouse gene assignments have been made including AK-1, LDHA, TRIP-1, HK, ACP-1, DIP-2. These studies provide extensive information on the mouse biochemical gene map and aid in our understanding of the evolution of the mammalian genome.

We have studied non-random segregation of chromosomes in hybrid cells. Of particular interest is the exclusion of mouse chromosome #11 and retention of #15 in Chinese hamster x mouse hybrids suggesting the presence of genes related to replication on these chromosomes. We are simultaneously comparing human hybrids made with normal and malignant cells to see if similar types of non-random segregation occurs. The hybrid cells thus are a test system for isolating certain chromosomes.

We have developed a computer data management system for use in somatic cell hybridization and gene mapping. This system is linked to NIH and will provide a means for rapid, efficient analysis of results and planning of new experiments.

We have mapped a human gene to chromosome 2 recquired for Aryl hydrocarbon hydroxylase (AHH)metabolism. This is part of a systematic study of genes related to metabolism of carcinogens and other xenobiotics. This was done in collaboration with Dr. H. Gelboin.

In collaboration with Dr. H. Rapp's lab, we have been testing the potential use of hybrid cells as immunotherapy agents. We are generating hybrids between human, rodent and Guinea pig hepatoma cells which will be used to immunize animals after surgical debulking. Hybrid cell techniques will provide methods for rapidily generating large numbers of cells for immunization after primary therapy of patients and present tumor agents to the patients in a new format. As a side benefit we have found that human lung cancer cells produce a growth factor(s) required for in vitro replication of the hepatoma cells.

We have tested the effect of primate tropic Type C viruses on the growth of human lung cancers in vitro and in nude mice. We find no effect.

Dr. Lalley of our laboratory has collaborated with Drs. Mukherjee and Schulman of NICHD in studies of chromosome transfer with phospholipid vesicles (Lipochromosomes). They have found for the first time the co-transfer of 3 different human X-linked genes using this system. This represent a major advance in mammalian somatic cell genetic techniques.

Dr. E. Kakpakova visited our laboratory as part of the USSR-USA exchange in cancer genetics and performed experiments using hybrid cells relating to malignancy. She brought with her special Djungarian hamsters for these experiments from her home laboratory.

In studies of the side effects of chemotherapy agents Dr. Gazdar has found that streptozoticin induces type C viruses in the pancreases of mice. This represents an important observation of SZ effect.

Dr. Bunn's laboratory has concentrated on studying the kinetics of human malignancies and megakaryopoiesis. Methodologies for human tumor cell kinetic studies have ben developed. Cytokinetic studies on patients with mycosis fungoides and the Sezary syndrome have shown sites of malignant cell proliferation, and demonstrated the slow rate of proliferation.

They have found malignant plasma cells have low labelling indcies, and that metastatic SCBC have labelling indices that vary greatly between the different metastatic sites in the same patients.

Megakaryopoiesis is being studied and evaluation of in vitro assays are being carried on to test patient status during chemotherapy. Model systems revealed colcemid not to reduce the thrombocytopenia of cyclophosphamide.

Dr. Cohen's laboratory has been investigating tumor blood flow in an experimental murine solid tumor system. The assays have been standarized and the stasis that exists in the tumor cell circulation is being experimentally modified by changing blood viscosity and anticoagulation.

Dr. Fossieck's laboratory has been developing and evaluating counterimmunoelectrophoresis (CIE) assays for the early detection of infection. Tests for S. pneumoniae, K. pneumoniae, H. influenzae, N. meningiditis, S. aureus, and C albicans are currently in use. Surveillance studies in a prospective fashion are being carried out and animal model systems developed.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 03024-08 VAMO

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Clinical Trials and Miscellaneous Clinical Investigations

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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(Continued)

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SECTION
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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
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CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this project is to study new methods of evaluating and treating malignant diseases. Therapeutic approaches include: combination chemotherapy, combined modality chemotherapy with radiotherapy, and immunotherapy. Intensive induction regimens, alternating combinations, low dose combination regimens, new agents (high dose methotrexate with citrovorum rescue, streptozoticin, ICRF 151, Ifosphamide, thymosin hemibody radiotherapy)are tested. The major cancers studied include: lung (small cell, epidermoid, adenocarcinoma and large cell carcinoma); hepatocellular gastric; prostatic; multiple myeloma; and mycosis fungoides-Sezary syndrome. New methods of evaluating response to therapy are explored with: fiberoptic bronchoscopy, peritoneoscopy, gastroscopy, and tumor cell markers. Complications of therapy are studied such as: fever, infection, interstitial pulmonary disease, metabolic and nutritional dearrangements. Clinical histopathologic correlations are made before and after therapy. Computer programs for storage, retrieval and manipulation of clinical research information are developed.

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(Rev. 10-76)

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2.0 CLINICAL TREATMENT STUDIES

2.1 SMALL CELL CARCINOMA

2.11 Combination Chemotherapy (CMC VAP VI Regimen) (Dr. Cohen and Senior Staff). (Protocol 75-4, 77-1)

The major advances made by the NCI-VA Medical Oncology Program during FY 77 involve improving the prospects for long duration, disease-free survival in small cell bronchogenic carcinoma (SCBC). Previous studies by our group have developed two active non-cross resistant drug combinations cyclophosphamide, methotrexate, CCNU (CMC) and vincristine, adriamycin procarbazine (VAP). Our current work has established several important principles in the treatment of SCBC. We have shown that intensive induction chemotherapy increases the overall response rate in SCBC and more significantly increases the complete response rate. The optimal degree of induction intensity has not yet been reached and ongoing protocols are increasing induction intensity. The toxicity of the induction regimen is acceptable and high dose chemotherapy is well tolerated by ambulatory patients and can be given on an open hospital ward without the need for prophylactic non-adsorbable gastrointestinal antibiotics. At present there are 4.7% deaths due to treatment complications during induction chemotherapy. We have demonstrated that the maximum response to a given drug combination in SCBC occurs in the first 6 weeks of treatment and early decisions as to treatment efficacy of a regimen can be made.

Consequently we tested the introduction of a new drug combination after 6 weeks of treatment with the inducing regimen. High dose induction therapy given for 6 weeks utilizes cyclophosphamide 1500 mg/M2 on days 1 and 22, methotrexate 15 mg/M2 twice weekly for 5weeks and CCNU 100 mg/M2 on day 1. (CMC) During weeks 7-12 patients receive adriamycin 60 mg/M2 and vincristine 1.4 mg/M2 on days 1 and 22 and procarbazine 100 mg/M2 on days 1 to 10 and days 22 to 31 (VAP). Maintenance dose of CMC are reduced to 500 mg/M2 for cyclophosphamide, 10 mg/M2 for methotrexate and 50 mg/M2 for CCNU. We found that adding the new noncross resistant regimen, VAP, doubled the complete response rate. We than asked in the same trial if a third combination would further help by randomizing the patients to continue on alternating cycles of CMCVAP or adding a third regimen VP16 (125 mg/M2 days 1,3,5,22,24,26) and ifosfamide (2400 mg/M2 on days 1, 2,3,22,23,24) (VI) for a CMC-VAP-VI program. No additional complete responses or increase in response durability were seen in patients receiving the three versus the two combination regimen.

In 64 patients entered into this trial the complete response rate at 6 weeks was 27% and at 12 weeks 52%. In patients with disease limited to one hemithorax including ipsilateral supraclavicular nodes the complete response rate at 12 weeks was 70% versus 40% for patients with disease outside of the thorax (extensive disease). This study program using intensive chemotherapy alone represents a unique trial in the USA in the treatment of SCBC. It yielded as good complete response rates and survival as trials using combined chemotherapy and radiation therapy even for limited disease. Most importantly, three of the first 12 patients treated are disease-free 20-24 months after initiation of treatment indicating the possibility for long term survival and cure in this disease.

2.12 Sites of Response and Relapse

The patients entering our SCBC trials are all carefully staged and the sites of response and relapse of SCBC have been determined leading to several important conclusions for future staging and treatment planning. Metastatic sites responding well to initial chemotherapy include lymph nodes and bone marrow, while pulmonary parenchymal involvement is less responsive, but more responsive than liver involvement. We have shown bone response is difficult to evaluate using bone scans alone and histologic confirmation of response is needed. The patients with supraclavicular lymph node involvement contralateral to the primary lesion behave as limited disease patients leading us to change the staging system. Our studies have shown that relapses occur in sites of bulky disease, particularly the chest. Our subsequent protocols test the addition of radiotherapy to our chemotherapy regimens. Treatment of disease in sanctuary sites such as the CNS is important in SCBC and our studies have shown that high dose nitrosourea offers a therapeutic alternative to prophylactic cranial irradiation with less than 10% of patients at risk developing CNS metastases. Future studies will combine high dose nitrosourea and prophylatic cranial irradiation to try to further reduce this incidence.

2.13 Combination chemotherapy CAPO Regimen (Dr. Minna and Senior Staff) (Protocol 77-2)

These trials have shown the dramatic differences in complete response rate, disease free and overall survival between patients with limited and extensive disease indicate the need for new induction regimens for extensive disease patients. In addition our current trials have demonstrated the durability of complete responses is inversely related to the time required to achieve the complete response. One of 17 patients obtaining a complete response in the first 6 weeks of treatment while 8 of 16 patients obtaining a complete response at a later date have relapsed. We are thus combining this and the previously mentioned information and have designed a new intensive induction regimen for extensive disease patients consisting of cyclophosphamide (1000mg/M2), adriamycin (60 mg/M2), vincristine 1.4mg/M2 and VP-16 150mg/M2 all given on days 1, 8 and 21, 28 in an attempt to rapidly achieve induction. This will be followed by our standard maintenance therapy of CMC-VAP.

2.14 Phase II studies: (Drs Bunn, Minna and Senior Staff)
(Protocols 76-5, 76-9)

Identification of active non-myelosuppressive drugs is very important in designing new induction and maintenance regimens for SCBC because of progressive hematologic toxicity. Three such possible agents are streptozoticin, high dose methotrexate (HDMTX) with leucovorum factor rescue, and cis-platinum. Phase II studies of streptozotocin at a dose of 100 mg/M2/week have failed to yield response in five patients thus far treated. Transient response have been seen with cis-platinum and none with HDMTX (12 gm/M2) in a few patients studied.

2.15 Hemibody Radiotherapy (Dr. Ihde) (Protocol 77-3)

SCBC is high responsive to radiotherapy. While extensive trials have been conducted of local radiotherapy we want to try using radiotherapy as a systemic agent. This involves a phase I-II study of hemibody radiotherapy. Patients will receive 200 rads/week for 4 weeks. If response are seen we plan to combine hemibody radiation with chemotherapy in untreated patients with disseminated disease. Together with Dr. F. Bilek (Chief Radiation Therapy WVAH) 4 patients with advanced, relapsing disease have been treated thus far in a pilot study and no response seen.

2.16 Treatment of Mixed Histology Patients (Drs. Minna, Bunn and Senior Staff). (Protocols 75-4, 77-1,77-2)

Histologic observations of proposed small cell patients have identified a group of patients with mixed small cell and non-small cell carcinoma. Ten patients who have this mixed picture usually SCBC with large cell anaplastic carcinoma) have been treated on our primary small cell carcinoma protocol (vide supra). There is no difference in initial response rates of mixed histology patients but response duration appears to be shorter than for patients having only small cell carcinoma. These studies are important since they will allow identification of another subset of lung cancer patients predictably responsive to chemotherapy.

2.16 Long Term Survivors

The most important thrust of our protocols is to obtain long term, disease free survivals in SCBC. We have begun to develop a registry for such patients in cooperation with Dr. F. Muggia and the CTE program. In our own experience, three of 23 patients treated in our first intensive chemotherapy protocol survived 2 years or more. One patient is in complete response for 4.5 years off of all chemotherapy for the past 2.5 years. One patient died in an auto accident at 2 years and was free of tumor at autopsy. A third patient had an isolated bone metastases at 20 months, received local radiation therapy and now appears to be in complete remission 3 years after starting therapy. Three of 12 patients at risk treated during the early part of our last trial are approaching 2 year disease free survival. Thus, a subset of potentially cured patients is developing.

2.2 ADENOCARCINOMA AND LARGE CELL ANAPLASTIC LUNG CANCER (Dr Minna and the Senior Staff).

The major problem in nonsmall cell lung cancer is to find active chemotherapy regimens.

2.21 High Dose Methotrexate (HDMTX) with Citrovorum Factor Rescue (CVF) (Protocol 76-9)

HDMTX has been proposed as a highly active regimen in non-small cell lung cancer potentially useful in adjuvant settings. However, it was found to be highly toxic in older patients. We have studied two treatment regimens. In the first, a 6 hour infusion with doses escalating up to 12 gm/M2 giving peak MTX levels of 10-2 M followed by CVF was evaluated in 14 patients. No objective responses were seen, and disease progression occurred during treatment in 3/4 of the patients. However, alkalinization, hydration and monitoring of MTX levels with a radioisotopic assay, toxicity was minimal. Thus, we find no evidence for activity of this regimen a fact extremely useful in deciding on proposed non-small cell lung cancer trials. In the second regimen we gave 30 hours of continous infusion at a dose to achieve levels of 10-5 M MTX followed by CVF. No responses have been seen in 7 patients studied. While this has been a negative study patient accrual is continuing. Because of the positive reports in the literature it is important to unequivocally establish whether or not the regimen has activity and could be given safely in adjuvant trials.

2.22 Ifosfamide and Ascorbic Acid. (Dr. Cohen). (Protocol 76-10)

Patients with tumor progression on HDMTX or who are ineligible for the HDMTX protocol were treated with ifosfamide 2400 mg/M2 daily for 3 days every 3 weeks and with ascorbic acid 10 gm/M2 orally. The rationale for the latter drug was to attempt to ameliorate ifosfamide bladder toxicity and possibly to stimulate the immune system. One objective response has been seen thus far in 4 patients.

2.3 EPIDERMOID LUNG CANCER (Dr. Ihde) (Protocol 75-1)

Seventeen patients with unresectable epidermoid carcinomas of the lung were treated with combination chemotherapy consisting of bleomycin, streptozotocin, and methotrexate, designed to minimize myelosuppression. Of great interest, objective responses in lung, lymph nodes or liver were seen in 7 patients (41%). This is distinctly different from our past experience. However, these responses were of short duration and in general were not associated with increased survival. Significant hematologic toxicity occurred in only 2 however both of these died from the toxicity. Of interest, the major toxicities observed were nausea, vomiting mucositis and a weight losing syndrome related to chemotherapy causing half the patients to lose 10 lbs or more of weight during the 6-week induction period. This study is now terminated and a new study is being designed.

2.4 GASTRIC CARCINOMA (Dr. Bunn and Senior Staff). (Protocol 76-8)

2.41 Our principal protocol for gastric carcinoma patients evaluates the sequential administration of two active drug combinations in patients with unresectable, measurable disease. Treatment consists of 5 flurouracil and MeCCNU alternating with adriamycin and mitomycin C. A major problem in evaluating the response to therapy is finding measurable disease. In addition to routine studies we use gastroscopy to follow patients. 15 patients have been entered. There are 2 responses (1 CR, 1 PR), 11 stable disease and two progressions. Ten patients are still alive. The longest remission duration at present is 10+ months. Patient accrual is continuing. Of importance, we have demonstrated that patients with surgical debulking of their disease have long term, good quality of survival suggesting that combined surgical and chemotherapy treatment by be tried in patients with far advanced disease.

2.42 Phase II Trials in Gastric Carcinoma: ICRF 159. (Protocol 76-17)

This phase II protocol evaluates ICRF 159 (previously reported to be active in other gastrointestinal malignancies) in patients with relapsing on the primary gastric carcinoma protocol with measurable disease. Five patients have been entered and one partial response seen.

2.5 PROSTATE CARCINOMA (Dr. Ihde and the Senior Staff.)

2.51 5-Flurouracil Versus CCNU.

Nineteen patients with metastatic prostate cancer who had failed hormonal therapy were randomized to one of the above drugs. Two of 8 patients on 5FU and 4 of 10 patients on CCNU had a partial response. The median response duration was 3.5 months for 5FU and 6.8 months for CCNU. Myelotoxicity induced by full doses of the above agents was mild to moderate and tolerated by these older patients. The study is terminated.

2.52 Combination Chemotherapy: Cyclophosphamide Plus Adriamycin, and Secondary Hormone Manipulation. (Protocol 76-6)

The efficacy of combination chemotherapy in hormone-resistant Stage D prostatic cancer and evaluation of newer means of assessing responses to therapy, always a difficult problem in prostate cancer, are being investigated Patients are treated with cyclophosphamide and adriamycin, two drugs with demonstrated single-agent activity in this dissease, and at disease progression are given a hormonal therapy to which they have not previously been exposed, either orchiectomy, diethylstilbesterol, stilphostrol, or medroxyprogesterone. Lymphangiography, CAT scans of the pelvis, and serial determinations of CEA and LDH isozymes are being studied during treatment. Objective responses to combination chemotherapy have been observed in 5 of 17 patients. Patient accrual is continuing. The myelotoxicity is mild to moderate and well tolerated by these older patients.

These studies demonstrate that prostate cancer is a disease responsive to chemotherapy and should form the basis for treatment of advanced disease patients failing hormone manipulation.

2.7 CUTANEOUS T CELL LYMPHOMAS: MYCOSIS FUNGOIDES-SEZARY SYNDROME (Dr. Bunn and the Senior Staff). (Protocol 76-12)

This protocol evaluates the staging and treatment of patients with cutaneous T cell lymphomas including mycosis fungoides and the sezary syndrome. Patients are staged by conventional studies and with peritoneoscopy. Patients with Stage I are randomized to receive electron beam (EB) treatment with or without low dose nitrogen mustard (NH2). Patients with more advanced stages are treated with combined modality therapy: EB and alternating cycles of vincristine, adriamycin, bleomycin (VAB) and cyclophosphamide, methotrexate, prednisone (CMP). In these studies we are exploring several principles; including combined modality therapy, use of NH2 as an adjuvant to EB, the use of low dose combination chemotherapy in this usually slow growing disease, and alternating cycle chemotherapy. Thus far 16 patients have been entered and all have responded with a high complete response rate and are alive with a median follow-up of 4 months. The major problem initially was gaining experience with EB therapy and combining this with simultaneous chemotherapy. In addition, in many instances it is difficult to make the histopathologic diagnosis and the skin, blood and lymph nodes of all cases are being studied with electron microscopy (Dr. Guccion). In addition our slides are reviewed by the National Mycosis Fungoides Study Group and we supply demographic information to this group. A variety of other collaborative studies are performed on these patients including: the study of the role of malignant T-cells as helper cells (Dr. Broder); study of cytogenetic abnormalities (Dr. Wang-Pheng); evaluation of the role of lymphangiography (Dr. Dunnick); T-cell quantitation and evaluation of the presence of malignant cells in the peripheral blood; (Dr. Schechter); study of the patterns of cell proliferation (Dr. Shackney and Brigham); the presence of steroid receptors on malignant cells (Dr. Thompson's lab); evaluation of antibody titers to staphlococci by counterimmunoelectrophoresis, a prevalent and serious infectious complication in this disease (Dr. Fossieck); and in collaboration with the MB (Dr. Fisher) the study of antithymocyte globulin in patients who have failed primary therapy. These protocol studies are continuing.

2.8 HEPATOCELLULAR CARCINOMA (Dr. Ihde and the Senior Staff).(Protocol 76-11)

Patients with surgically unresectable hepatocellular carcinoma are being treated with a combination of adriamycin, a drug with reproducible activity against this tumor, and streptozotocin, a non-myelosuppressive agent which can be combined with adriamycin in full doses. (Protocol 76-11). Serial determinations of alpha fetoprotein during treatment are being performed by Dr. K.R. McIntire, and gallium scans are being investigated for their utility in detecting metastatic disease and following response to therapy. One of 7 patients (14%) has had a prolonged objective response to treatment, but is the only good risk (full ambulatory and no jaundice) patient thus far studied. This and our previous studies emphasize the types of patient with hepatocellular carcinoma likely to benefit from treatment.

Of importance several young patients from previous trials are continuing off adriamycin herapy with stable disease and excellent quality of life for long periods of time. Patient accrual is continuing.

2.9 CLINICAL HISTOPATHOLOGIC CORRELATIONS: (Drs. Matthews and Gazdar)

2.91 Correlation of Clinical Response with Subtypes of Small Cell Carcinoma.

Initial diagnostic material on 156 patients with SCBC treated with chemotherapy or combined modality chemo-radiotherapy have been classified into three subtypes of SCBC: Lymphocyte-like, intermediate, and mixed lymphocyte like-intermediate groups. The subtypes classification were correlated with clinical responses to therapy. We have found tumors in all subtypes have shown objective responses. This re-emphasizes the importance of review of all lung carcinoma diagnosis by a pathologist experienced in distinguishing small cell from non-small cell lung cancer since the intermediate and mixed group can be mistaken for non-small cell lung cancer. The fraction of complete responders appears to be lower in the mixed and the fraction of non-responders higher in the intermediate and mixed group. Thus, subtyping may become important in planning therapy for SCBC.

2.92 Correlation of Bulk of Tumor and Sites of Disease at Autopsy in SCBC

Ninty-one autopsies of patients treated on protocols with SCBC have been reviewed. Combined modality therapy appears to decrease the amount of disease remaining in the chest. Comparsion of necropsy material from patients receiving moderate and intensive dose chemotherapy is ongoing. These clinical pathologic correlations are essential for evaluating the effect of treatment and trying to design new regimens.

2.93 Development of Non-Small Histology in Patients with SCBC.

Sixty-two patients with histologically confirmed SCBC have had sequential bronchial biopsies and cytologies during their treatment courses. Ten percent have shown the development of either in situ epidermoid carcinoma or frankly invasive epidermoid carcinoma. Cytology specimens showed changes occurring 6-9 months after the onset of therapy ranging from severe dysplasia which we now associate with chemotherapy, to frankly neoplastic squamous cells. In necropsy specimens we have found marked alteration in cell type including evolution of the tumor into: squamous cells, anaplastic large cells, tubule formation, or formation of multinucleated giant cells. In 2/91 patients no small cell tumor was identified but well differentiated epidermoid carcinoma with widespread metastases was found. Whether these changes represent separate primaries, treatment related changes, tumor induction, or change in pattern of differentiation is not known at present. However, we stress that these findings add a new complexity to the problem of SCBC treatment. Of great interest, while we have often seen small cell carcinoma become associated or differentiate into other cell types the reverse situation is not seen. This suggests a schema for the program of differentiation of bronchial epithelial cells.

2.95 Gastric Cytologies

In a manner analogous to the study of sequential bronchoscopy biopsies and cytologies in lung cancer we have been following patients with gastric carcinoma on our protocols before treatment, at the time of objective response and progression with endoscopically obtained gastric biopsies and cytologies. We are trying to correlate the response to therapy and other associated changes that might take place following chemotherapy. In addition, we are correlating gastric washing CEA levels with morphologic changes seen in these patients.

2.95 Histopathologic Correlations of Gastric Carcinoma Surgical Specimens.

Surgical specimens from 45 patients who have undergoing gastric resection as part of VA Surgical Adjuvant Groups protocols are being reviewed. The tumors are being classified into: intestinal type, diffuse type, mixed types as well as for host response and correlated with clinical course: rapid tumor progression, slow progression, and patients free of disease after 3 years.

2.96 Analysis of Autopsy Specimens in Patients Dying 1 month after Surgical Resection of Lung Cancer

Some of the most valuable data on lung cancer staging has come from an ongoing analysis of 235 VA Surgical Adjuvant Study patients who had surgical resection of the lung cancer and died within one month of operation. To date the following correlations have been made: one-third of patients following a curative resection had residual disease at autopsy that in the majority of cases was extrathoracic. This was particularly true for adenocarcinomas regardless of size, pleural or lymph node metastases, while large cell tumors regardless of size, pleural or lymph node involvement showed minimal tendency to metastasize despite of the bulky nature of the majority of tumors. Epidermoid tumors occupied an intermediate position. Of special note, were small peripheral epidermoid carcinomas that had pleural invasion that were found to have metastasized widely. These studies stress the different biologic behavior of the different histologies of non-small cell lung cancer which must be taken into effect in planning treatment regimens.

2.10 STAGING PROCEDURES

2.101 Fiberoptic Bronchoscopy (FOB) in Lung Cancer (Dr. Ihde)

Patients with non-small cell and small cell carcinoma are bronchoscoped initially, at set time intervals and at times of objective response or progression with bronchial washings and biopsies obtained to evaluate the utility of this staging procedure in the treatment of advanced lung cancer. We have found FOB to be extremely valuable in obtaining visual and histopathologic evidence of objective disease response and progression. 70 patients with small cell carcinoma have been studied in detail during treatment with alternating regimens of combination chemotherapy. Bronchoscopy revealed evidence of tumor in the chest in over 90% of patients prior to treatment, and in 7% this was the only evaluable chest disease. During therapy, over 30% of patients thought to be in complete response by chest X-ray criteria had evidence of residual disease at bronchoscopy, and the short-term relapse rate in the chest was much higher in these patients. Almost one fourth of episodes of progressive disease in the chest were first detected soley by FOB. Because of the much lower response rates to therapy, including very low complete response rates, FOB has not yet proven to be as useful in following the treatment of non-small cell lung cancer patients, but in some instances evidence of response or progression not apparent from the chest X-ray was obtained. The bronchial washings also provide large numbers of cells for clinical-laboratory correlations. In particular we have been performing cell kinetic studies with in vitro labeling of cells obtained at bronchoscopy. In addition, in cooperation with Dr. C. Lawrence, Metabolism Branch, NCI, the frequency of receptors for cytophilic antibody on pulmonary macrophages in patients with SCBC before and after treatment is being studied. These staging studies form the foundation for objective evaluation of all future treatment trials of advanced lung cancer.

2.102 Peritoneoscopy (Dr. Bunn)

This procedure is being evaluated as a staging technique for patients with small cell bronchogenic carcinoma, cutaneous T cell lymphomas, gastric carcinoma, hepatoma and other solid tumors.

2.103 Evaluation of Tumor Markers with Therapy.

We are attempting to correlate bulk of disease, response to therapy and early detection of disease progression through the sequential evaluation of tumor markers during a patient's course. Small Cell Bronchogenic Carcinoma (SCBC): In cooperation with Drs. .K. Becker and O. Silva, Endocrinology Section, WVAH it has been determined that calcitonin is elevated in 70% of SCBC patients with or without bone metastases. Calcitonin levels were found to fall to normal levels with the obtainment of a complete response and to rise at the time of disease progression. This is potentially a very useful marker in determining the adequancy of treatment of SCBC.

Gastric Carcinoma : We have found 100% of gastric cancer patients have elevated CEA levels in their gastric washings. Levels in these washings are higher than are blood levels. 25% of patients with benign gastric disease also have elevated CEA levels om their washings. In cooperation with Drs. Widerlite and Cohen, of the Gastroenterology Section, WVAH we are evaluating the relationship of blood and gastric washing CEA with disease status and response to therapy.

Prostatic Carcinoma: CEA was found to be elevated in most patients with prostatic carcinoma. However, we find that CEA levels do not parallel the clinical course of disease. Human chorionic gonadotrophin (HCG), human placental lactogen (HPL) and prostatic alkaline phosphastase (PAP) are rarely elevated.

2.11 INFECTIOUS DISEASE STUDIES (Dr. Fossieck)

2.111 Study of Fever in Patients with Lung Cancer and other Solid Tumors.

We have found that patients with solid tumors undergoing intensive induction chemotherapy develop marked granulocytopenia, and fever but infrequently have infection that can be documented. Often these patients can be observed without instituting broad spectrum antibiotic coverage. We are reviewing these findings and developing criteria for the clinical approach to this problem which appears to be a general one in patients with solid tumors in association with more aggressive chemotherapy.

2.112 Study of Interstitial Lung Disease in Cancer Patients.

We have found that patients with solid tumors undergoing intensive chemotherapy have developed interstitial lung disease of multiple etiologies including opportunistic infections, allergic reactions, metastatic tumor and as yet unknown processes. These patients present a different challenge with respect to age, associated medical conditions, as well as to neoplastic processes involving the lungs than patients with hematologic malignancies. In cooperation with Dr. P. Schaffer of the Surgical Service WVAH, and Dr. S. Spagnola of the Pulmonary Section, WVAH we are establishing criteria for the management of these difficult clinical problems.

2.113 Specific Infectious Disease Problems in Patients with Solid Tumors. Gram positive coccal bacteremia has occurred in 45 patients with solid tumors and accounts for approximately 1/3 of all bacteremias in our unit. Because this has not generally been recognized as a major clinical problem we are reviewing the predisposing factors, therapy and outcome of this complication. Gram negative bacillus bactermia has occurred in 65 patients with solid tumors and while this is widely recognized as a clinical problem in hematologic malignancies, we are seeing this problem in solid tumor patients without granulocytopenia. We are thus reviewing our data for clinical correlates and response to treatment. Pneumocystis carinii pneumonitis which previously has not been reported to occur in lung cancer patients has developed in 4 patients often not in association with corticosteroid therapy. Other unusual infections such as persistent Salmonella enteriditis in a patient with hepatocellular carcinoma in the face of appropriate antimicrobial therapy is being reported.

2.114 Clinicopathologic Correlates of Candida Infections in Cancer Patients. (Dr. Ihde)

Clinical records and autopsy protocols of 168 consecutive cancer patients dying with histologically documented candidiasis at the NCI were studied in collaberation with Drs. R. Young, W. McGuire, of the MB, NCI and Dr. H. Brereton, ROB, NCI and Dr. W. Roberts, Pathology Section NHLBI. The patient were classified into local and disseminated disease, clinical features predictive of disseminated disease in this population were ascertained, and pathologic features of major organ involvement investigated.

2.12 IMMUNOTHERAPY STUDIES (Dr. Cohen) (Protocol 76-4)

Patients receiving induction therapy for small cell carcinoma have been randomized to receive twice weekly thymosin 20 or 60 mg/M2 IM or a placebo for 6 weeks in collaboration with Drs. Chretien and Kenady, NCI. Side effects of thymosin so far seen include only local reactions. We have not seen any significant therapeutic benefit. However, in vitro immune function tests show that thymosin partially restores immune reactivity. This is an important study because of its randomized nature and because it represents the only trial of thymosin in solid tumor patients receiving only intensive chemotherapy and no radiotherapy. The study is continuing.

2.13 COLCEMIDE FOR THROMBOCYTOPENIA IN PATIENTS RECEIVING CHEMOTHERAPY (Dr. Bunn) (Protocol 76-13)

Patients with solid tumors receiving chemotherapy on protocols have been given alternating cycles of therapy plus colcemide to evaluate the effect of this agent in protecting patients against chemotherapy induced thrombocytopenia. The study is continuing.

2.14 DEVELOPMENT OF COMPUTER PROGRAMS FOR CLINICAL STUDIES

A computer terminal linking the NCI-VA program with the computer facilities at the NIH has been installed. A professional consultant (Mr. L. Berman) has been hired to establish programs for entering clinical data. Emphasis has been placed on developing a computerized flow sheet record that will provide continuing patient updates to the physicians responsible for patient care and slots to fill in relevant information obtained on each patient visit. The staging and other followup information (eg. biopsies, scans, X-ray studies), data on exact chemotherapy or other treatment given, are entered with a patient ID code and date and then the computer sorts and intergrates the information into a record that will be useful for patient care and protocol review. We feel such a system will greatly aid in the clinical research studies and meet the increased requirements for reporting to clinical research committees.

2.14 LITERATURE REVIEWS OF LUNG CANCER TREATMENT TRIALS

During the past year, invited review articles have been prepared on lung cancer diagnosis, staging, histopathology of lung cancer and the effect of single agent chemotherapy of non-small cell lung cancer, combination therapy and combined modality therapy of small cell lung cancer, and on randomized treatment trials in lung cancer.
While such reviews had been conducted in the past, these summaries have revealed new perspectives in lung cancer treatment: for example much of the reported single agent data in non-small cell lung cancer was overly optimistic and had to be reinterpreted. Objective response rates of 5-10% or less are likely to represent the true treatment responses in non-small cell lung cancer. Randomized trial data suggest that combination chemotherapy in non-small cell carcinoma is generally not significantly better than single agent trials. Current status in small cell carcinoma is generally summarized in paragraph 2.1 .

2.6 MULTIPLE MYELOMA(PROTOCOL 76-7) (Dr. Bunn and Senior Staff)

We have been experimenting with low dose alternating combination cycle chemotherapy in multiple myeloma. Following remission induction with melphalan and prednisone a four drug combination of vincristine cyclophosphamide, adriamycin and prednisone is given in an attempt to prolong remission and prevent drug resistance. 15 patients have been enetered with a median follow-up of 7 months. 12 patients have responded 2 have had progressive disease, and one is too early to evaluate. 13 patients are alive.

Collaborative studies are in progress in the following areas: Dr. G. Schechter, Hematology Section, WVAH is studying the role of osteoclast activating factor in the bony abnormalities of myeloma. She is also evaluating the bone marrow reserve of these patients following the acute administration of prednisone. Drs. Bunn, Nugent and O. Alibaster are studying cytokinetic changes in the tumor and normal bone marrow before and after chemotherapy. Dr. M. Potter, NCI is attempting to grow human myeloma cells in nude mice primed with mineral oil. The study is continuing.

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TITLE OF PROJECT (80 characters or less)

Laboratory Investigation of Tumor Cell Biology

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
21	11	10

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project uses a multidisciplinary approach to study tumor cell biology, so as to further our understanding of the basic nature of human malignancy, and to develop techniques for early detection and treatment. Our major effort is a genetic analysis of malignancy using somatic cell hybrids, and tumor virology. Interspecific hybrid cells are formed by cell fusion and analyzed by isozymes chromosome banding, and then tested for properties related to malignancy. These include: RNA tumor virus replication, spontaneous and induced expression of covert genomes, viral receptors, host range and biohazard potential, detection of virogenes by molecular hybridization, expression and regulation of cell and virion DNA polymerase, chromosome segregation, growth in nude mice, contact inhibition, expression of differentiated function, carcinogen metabolism including aryl hydrocarbon hydroxylase, and tumor antigens with immunotherapy trials. Correlation of chromosome content with these properties allows identification and subsequent gene assignment. Other areas include growth of human tumors in nude mice for cell kinetic and therapy trials, and studies of tumor blood flow and perfusion in experimental tumors.

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3.0 SUMMARY OF LABORATORY STUDIES NCI-VA MEDICAL ONCOLOGY PROGRAM

3.1 OVERVIEW

From the clinical study of solid tumor patients and their treatment trials, the investigators of the NCI-VA Medical Oncology Branch have developed several questions which they are asking in the laboratory. It is clear that fundamental questions of tumor cell biology must be answered in order to develop new approaches to cancer cause, prevention and treatment. Toward this end, we are attempting to develop clonal cell lines directly from human tumors in vitro and in immunosuppressed mice. These tumors are being particularly studied for their growth factor requirements and ability to differentiate. While hormone and growth factor requirements for breast and prostate cancer are partially understood, virtually nothing of the factor requirements for lung cancer is known.

In the case of small cell bronchogenic carcinoma, tumor cells replicate rapidly in the host but when placed in rich tissue culture medium fail to grow at all in the majority of cases and when lines are developed they double at a slow rate with much cell lysis. This observation is of fundamental importance. Clearly the patient is supplying some growth factor (s) which are not present in correct amounts in tissue culture medium. We are studying the requirements for nerve growth factor, epidermial growth factor and other hormones as well as medium conditioned by lung cancer cells of other histologic types and normal pulmonary supporting cells. Once these factors are defined a new approach to lung cancer therapy regulating growth factors can be undertaken. Similarly we are studying the differentiation of human tumors particularly lung carcinomas to determine at which steps of differentiation they are blocked. We are trying to overcome this block and develop methods for making lung cancers differentiate which could then be applied to patients. The lung cancer lines or hybrid cell derivitives will also be useful immunotherapy trials in patients.

Using somatic cell hybrid techniques we are trying to identify genes responsible for generating or suppressing malignancy, processing carcinogens, and oncogenic viruses. These include genes human tumors, and the genes required for RNA tumor virus replication and function. In related studies of fundamental biologic importance we are studying the evolution of human gene linkage groups in comparison to other species using hybrid cells.

In model tumor systems in animals we are studying tumor blood flow, cell kinetics and the effect of new modes of therapy such as addition of hyperbaric oxygen. In samples taken directly from patients, and in the tumor lines we are studying cell kinetic properties. The effects of chemotherapy agents as potential carcinogens and activators of oncogenic viruses is also studied.

3.2 CULTURE OF HUMAN TUMOR CELLS IN VITRO (Gazdar, Carney, Stull, Simms)

Tumors from patients with lung cancer, as well as tumors with certain differentiated functions are being established as continuous cell cultures.

Fresh tumor material is disperesed and plated in enriched medium (F14). From approximately 60 tumors, we have established 7 continuous lines. Several others have been subcultured multiple times, but are not fully established.

Two lines have also been established from small cell carcinomas of the lung. In contrast, over 20 specimens have not developed into continuous lines and are frozen in a viable state for future growth factor studies. One line grows in suspension, while the other grows as polygonal cells that adhere to the substrate. The population doubling time is slow (4-5 days), but the cell coubling time is probably much shorter, as considerable cell lysis is observed. The cells require a factor(s) elaborated by certain non-transformed cell for optimal growth. Studies of small cell differentiation have revealed a line with high histaminase activity. On electron microscopy, many membrane bound secretory type granules are present. Formaldehyde vapor exposure induces particulate cytoplasmic fluorescence, indicative of catecholamine synthesis and storage. The cells do not elaborate glucagon, insulin or calcitonin. We are currently doing detailed cell kinectics, and further biochemical characterization. The effect of differentiating agents (DMSO, CAMP, nerve growth factor), and characterization of the growth factor requirements is being pursued. Once fully established and characterized, the various cell lines may be used to develop in vitro and in vivo (in nude mice) models for chemo- and immunotherapy. Studying the growth factor requirements of tumor cells may provide insights into regulating their growth in cancer patients.

Two lines have been established from mycosis fungoides lymph nodes, but only one has been characterized. It grows in large aggregates in suspension culture. Most of the cells produce membrane bound immunoglobulins (B cells), but a subpopulation forms rosettes with sheep red cells (T cells). Terminal transferase activity is absent. The cells proliferate rapidly when inoculated intracranially in nude mice.

A gastric carcinoma was established after passage of the original tumor material in nude mice. It consists of tightly knit epitheloid cells containing mucin. The cells rapidly induce subcutaneous tumors in nude mice. A pleural mesothelioma, and pulmonary large cell carcinoma have also been established in long term culture, but their characterization is still in progress.

3.3 GROWTH OF HUMAN TUMORS IN NUDE MICE

A nude mouse colony has been established in a clean, limited access room. The mice, of BALB/c background, are obtained under NIH contract from Charles River Farm. They arrive in poor shape, often infected with pneumonia or hepatitis viruses, and seldom live longer than 4 months. Over 100 human tumor samples have been placed in nude mice but transplantable tumors only occasionally established. This is probably due to the short nude mouse life span in our lab at present. However, several new human tumors of interest have been serially passaged in nude mice. These include small cell carcinomas and large cell carcinomas of the lung, melanomas, gastric and colon adenocarcinomas, mycosis fungoides nodes, pleural mesothelioma, and sweat gland carcinoma.

Histologically, the transplanted tumors closely resemble the parent tumor. The oncogenicity and tumor type of in vitro human lines and interspecies hybrid cells are also tested in nude mice. The tumors in nude mice are used for a) asource of human tumor material, b) establishment of in vitro lines, c) models for chemo,radio,and immunotherapy, d) cell kinetic studies, e) studying the genetics of malignancy, f) studying the differentiation and growth factor requirements.

3.4 SEARCH FOR HUMAN GENES CODING FOR MALIGNANCY IN HYBRID CELLS (Moss, Carney, Edgell, Minna)

Since on the cellular level, malignancy is a heritable trait, it seems probable that specific, potentially identifiable aberrations in the genetic control mechanisms for cellular proliferation exist. To study these control mechanisms we are attempting to produce somatic cell hybrids between malignant human tumors and benign diploid animal somatic cells which will segregate the chromosomes of the malignant human parent. We can then study these cells for genetic markers and for the expression of malignant growth characteristics both in tissue culture and in the athymic nude mouse. Hopefully, these experiments will yield information as to the existence of specific chromosomes required for malignancy in human tumors. With this information more detailed analysis of the specific "oncogenes" involved could be carried out.

As an alternative approach to the problem of genetic control of malignancy somatic cell hybrids between tumor lines and benign diploid cell lines which segregate the chromosomes of the benign parent are being studied. In these hybrids we are looking for the presence of the normal parent that could implement the malignant genome and thus suppress malignant expression in the hybrids. If this occurred, we should see both "malignant" and "benign" hybrids produced, depending on which of the chromosomes of the benign parent were retained. Our results to date from a large number of hybrids between Chinese hamster lung tumor cells and normal mouse spleen cells have excluded all mouse chromosomes but #11 as being able to suppress malignancy when present as a single copy. These experiments are being extended to look not only at the presence or absence of specific chromosome markers, but also to analyze the quantitative relationships between the chromosomes in the "malignant" hybrid cell lines to assess the effect of "gene dosage" on malignant expression.

3.5 IDENTIFICATION OF MOUSE GENES REQUIRED FOR MURINE LEUKEMIA VIRUS REPLICATIONS (Gazdar, Oie, Lalley, Moss, Minna)

Interspecies hybrid cells formed by the fusion of permissive with non-permissive parent cells are useful tools to study the cellular factors regulating virus regulation. We tested the replication patterns of several ecotropic and amphotropic strains of murine leukemia virus in primary Chinese hamster x mouse hybrid cells segregating mouse chromosomes. The viruses replicated in mouse and some hybrid cells, but not in Chinese hamster cells, indicating that virus replication requires dominantly expressed mouse cellular genes, which are not present in hamster cells. The replication patterns of all of the ecotropic strians in the hybrid clones were similar with one another while the amphotropic virus replication patterns were also similar with each other. However, when compared the replication patterns of these two virus classes were dissimilar, indicating that they require different mouse chromosomes for replication. Chromosome and isosyme analyses assigned a gene, Rec-1, to mouse chromosome 5 that is necessary and may be sufficient for ecotropic virus replication. Similarly, our data indicate that mouse chromosome 8 carries a gene, Ram-1, which is necessary and may be sufficient for amphotropic virus replication. Because chromosome 19 tended to segregate with chromosome 8 in these hybrids, we cannot confidently exclude 19 as being independent of amphotropic virus replication. Hybrid cell genetics confirm the assignment of the Fv-1 locus to chromosome 4 previously made by sexual genetics. In addition, our results demonstrate that hybrid cells by segregating chromosomes may permit viruses to replicate that are restricted in both perental cells.

A study of the binding of Rauscher leukemia virus envelope glycoprotein (RLV gp71) and murine leukemia virus replication in the hamster x mouse hybrid cells showed that RLV gp71 binding to hybrid ce-ls correlated with the replication of murine ecotopic viruses. No correlation was observed between RLV gp71 binding and amphotropic virus replication. Therefore, all ectropic viruses use the same cell receptors for adsorption to cells which differ from that used by amphotropic viruses. Chromosome and isozyme analyses showed that the Rec-1 gene, previously assigned to mouse chromosome 5, was necessary for RLV gp71 binding to hybrid cells. The data suggest that Rec-1 determines the expression of specific cell surface receptors required for ectropic virus adsorption to mouse cells.

3.6 SPONTANEOUS EXPRESSION OF TYPE C VIRUS IN HYBRID CELLS (Park, Oie, Gazdar)

All mouse cells contain one or more genomes for strains of murine leukemia virus (MuLV). Because there are major mouse strain differences in the spontancous expression patterns, we studied spontaneous expression of MuLV in hamster x mouse hybrid cells formed by fusing the continuous Chinese hamster cell line E36 with normal mouse spleen or bone marrow cells from several strains. All the hybrids segregated mouse chromosomes allowing one to test for the gene requirements for virus activation or repression. Expression in the hybrid cells and in control mouse spleen cultures was followed by DNA polymerase and fluorescent antibody studies. Major differences were found between the 4 mouse strains studies. In AKR mice, virtually all of the spleen and hybrid cultures released virus, while none of the NIH Swiss mouse cultures or hybrid clones were positive. Most of the NZB spleen cultures were positive, while relatively few of the hybrid clones released virus. The opposite situation was present in the BALB/c mice: none of the spleen cultures were positive, while several of the hybrid clones released virus. There were also differences in the types of MuLV strains released by the different mouse strains: AKR mice released N-tropic virus, while NZB mice released xenotropic virus. BALB/c hybrid cultures released both N- and xenotropic viruses. Virus expression occured independently of the mouse chromosomes containing the Akv-1, Fv-1 and Fv-2 loci. The cell passage level also influenced virus release from BALB/c hybrid cells. In some hybrids visur release occured only after several passages, suggesting that it may have been associated with loss of a mouse chromosome containing a gene for a 'repressor' substance. One positive clone was subcultured extensively in an unsuccessful attempt to get virus negative subclones. Chromosome studies showed that one positive subclone retained only mouse chromosome 2 translocated to a hamster chromosome. Thus we have demonstrated that mouse chromosome 2 can serve as a potential integration site of MuLV. By isozyme and chromosome studies, we are attempting to map the mouse virogenes, as well as any repressor substances preventing spontancous virus release. We are also determining whether the MuLV virogene can transfer into the hamster genome within the hybrid cells.

3.7 IDENTIFICATION OF HUMAN GENES REQUIRED FOR PRIMATE TROPIC RNA TUMOR VIRUS REPLICATION

Several approaches are being taken in an attempt to identify human genes coding for Primate-tropic RNA tumor virus replication using somatic cells hybrids. In the first, somatic cell hybrids formed between rodent (mouse and Chinese hamster) cells and human cells containing different human chromosomes are infected with different types of primate tropic Type C Viruses: Baboon, M7; Wooley Monkey, SSV; Gibbon Ape, GALV; cat CCC and FELV; mouse amphotropic and xenotropic. In the second, human cells are first preinfected with the primate tropic viruses and then fused to rodent cells. In both experiments, the human chromosome composition is determined and correlated with virus expression in the hybrids. Genes coding for receptors, integration sites, and other genes required for replication, or viral gene maintanence are determined. Similarly genes that would be deleterious for cell growth in the presence of the virus are selective excluded.

3.71 ASSIGNMENT OF A GENE TO HUMAN CHROMOSOME 6 FOR BABOON M7 VIRUS REPLICATION (Lemons, O'Brian and Sher)

Although this project in major part was started in the NCI-VA MOB it was completed under circumstances protested by the Chief of the NCI-VA MOB in the Lab of Viral Carcinogenesis. Since this represented a major commitment of NCI-VA effort the results are briefly described. Mouse x human and Chinese hamster x hyman hybrid cells were infected with Baboon M7 virus and virus positive and negative clones identified by DNA polymerase and radioimmunoassays. These were correlated with isozyme analyses and revealed an association in the particular hybrids tested with human chromosome 6. Subclones of virus positive hybrids were prepared and positive and negative subclones for virus identified. Isozyme analysis again revealed an association with human chromosome 6. The data from these sets of hybrids suggest genes required for replication, maintainence or integration may be present on human chromosome 6.

3.72 SEARCH FOR HUMAN GENES REQUIRED FOR BABOON M7 AND WOOLEY MONKEY SSV VIRUS IN HYBRID CELLS (Brown, Oie, Gazdar, Minna)

Hybrid cells between Chinese hamster and human patients with acute and chronic leukemias were tested for their ability to support the replication of Baboon (M7) and Wooley Monkey (SSV) virus using DNA polymerase and flourescent antibody assays. Positive and negative hybrid clones were detected for M7 virus. A large number of human chromosomes can be excluded as necessary or sufficient. At present it appears that in these hybrids human chromosome 6 is neither necessary or sufficient for M7 replication. The analysis of SSV replication in hybrid cells is more complicated because SSV also replicates in a low percentage of parent rodent cells. However, in the first 3 weeks, hybrids can be divided into two classes: those replicating SSV in 1-2% of cells (like the Chinese hamster parent) and cells replication 10-40 fold more virus. The chromosomes of these two classes are being compared to determine if specific human genes are involved or whether any of the number of human genes can serve to increase the replication capacity of the hybrid cells.

3.73 TYPE C VIRUS EXPRESSION IN MOUSE X PREINFECTED HUMAN HYBRID CELLS

(Gazdar, Edgell, Oie, Lalley, Minna)

We are attempting to map the integration sites of three type C viruses by fusing preinfected human cells with "non-permissive" mouse cells. Baboon M7, feline xenotropic (CCC) and Wooly monkey nontransforming component (SSV) viruses readily infect human but not mouse cells. A thioguanine resistant clone of A549, a human lung adenocarcinoma cell, was developed and sublines of it were chronically infected with the three viruses and subcloned. The virus infected subclones and control uninfected cells were fused with bromodeoxyuridine resistant 3TE-4E mouse cells, and resultant hybrid clones selected in HAT-supplemented medium. 3T3-4E cells were used because they do not release type C virus spontaneously or after chemical induction. About 20-40 hybrid clones were selected from each fusion, and grown to mass culture. Isozyme and cytogenetic studies indicated that all the hybrid clones were segregating human while retaining mouse chromosomes. The clones were tested for virus production by RNA-dependent DNA polymerase and fluorescent antibody assays. Most of the infected clones continued to release small amounts of virus and had polymerase levels that were 0.1 - 3% of the control values. A few clones were free of detectable virus release. In contrast, uninfected hybrid clones did not release virus. The apparently virus-negative M7 infected clones were co-cultivated with A549 cells. When each of the "virus negative" hybrid clones were co-cultivated, each of the "virus negative" hybrid clones were co-cultivated, M7 virus could readily be detected 7-21 days later. These studies demonstrate that (a) the mouse genome suppresses production of all 3 viruses in the hybrid cells, and (b) the M7 virogene is not completely eliminated as human chromosomes are lost.

To further understand the nature of resistance of mouse cells to M7 infection, chronically infected A549 cells were co-cultivated with 3T3-4E cells for 28 days and the mouse cells subsequently selected for in bromodeoxyuridine supplemented medium. All mouse clones so obtained released low but detectable amounts of M7 virus, which were rapidly amplified by co-cultivation with A549 cells. Thus the apparent resistance of mouse cells to M7 infection is an artifact of the detection system. There is little or no resistance at the entry or integration levels, but virus output is restricted. Certain endogenous xenotropic viruses may be under similar control mechanisms, and our experiments may explain how trans-species spread of xenotropic viruses occurs.

3.8 MAPPING OF HUMAN GENES USING HYBRID CELLS (Brown, Lalley, Minna)

Man x rodent somatic cell hybrids possess three characteristics which make them suitable for human gene mapping experiments. First, these hybrids preferentially lose human chromosomes thur, stimulating genetic segregation and making it possible to isolate clones with varying numbers and combinations of human chromosomes. Second, because of evolutionary differences several enzymes markers are available for genetic analysis. Third, chromosome banding techniques make it possible to identify specific human chromosomes thus allowing for the assignment of genes to specific chromosomes. Employing this experimental system, the linkage relationships of peptidase S (PEPS) and peptidase D (PEPD) in man were investigated. It was found that PEPD segregated concordantly with GPI and therefore the gene coding for PEPD can be assigned to chromosome 19 in man. This confirms the provisional assignment of PEPD reported previously by McAlpine et al. PEPS segregated concordantly with PGM-2. Since PGM-2 is assigned to chromosome 4 in man, the gene coding for PEPS can also be assigned to chromosome 4. It is interesting to note that PEPS and the homologous enzyme of PGM-2 in the mouse (PGM-1 in the mouse), were also found to be linked and are located on mouse chromosome 5 indicating that this linkage group is conserved between man and mouse. In fact, the mouse linkage data were used to predict the chromosomal assignment of human PEPS thus indicating the predicative value of comparative gene mapping. It should also be pointed out that the human genes coding for 3 other peptidases (PEPC, PEPB, and PEPA) have been assigned to three different chromosomes. Together with our data this indicates that there is no clustering effect of genes coding for five enzymes of similar function, the peptidases. This data aids in our understanding of the functional organization of the human genone.

3.9 EXPRESSION OF DIFFERENTIATED FUNCTIONS IN LUNG CANCER HYBRIDS

A cell line (A549), established from a human bronchiol-alveolar cell carcinoma, elaborates multilamellar bodies with the biochemical properties of surfactant. A thioguanine resistant subline was fused with mouse B82 cells and the resultant hybrids isolated in HAT supplemented medium. Electron microscopy revealed that the hybrid clones were of 3 types: - a) those with many multilamellar bodies, b) those with only a few, and c) those without any bodies. Isozyme studies confirmed that the hybrids were segregating human chromosomes. These studies indicate that surfactant production is expressed dominantly in hybrid cells, and that it is theoretically possible to map the genes required for its production.

3.10 COMPARATIVE GENE MAPPING IN MOUSE, CHINESE HAMSTER AND RABBIT (Lalley, Moss, Minna)

It is now possible to generate somatic cell hybrids which preferentially lose the chromosomes of any desired species. Therefore, this parasexual technique used so successfully in human gene mapping can be utilized to map genes of other species. This provides the opportunity to investigate the linkage relationships of homologous genes in several species. Data derived from such studies will help determine which linkage groups have been conserved or disrupted during evolution thus aiding in our understanding of the mechanism of the evolutionary process itself.

Using the somatic cell hybrid approach 3 sets of hybrids have been constructed which segregate mouse, Chinese hamster or rabbit chromosomes respectively, and a genetic analysis of over 25 different genes was initiated. The linkage data obtained from these studies was then compared to the human gene map. The mouse gene mapping was pursued first since the mouse is better understood genetically and is an important animal model system for cancer studies. Genes coding for the enzymes 6-phosphogluconate dehydrogenase, enolase, phosphoglucomutale 1 and adenylate kinase - 2 have been assigned to the short arm of human chromosome 1. Our data assigns all four of these genes to chromosome 4 in the mouse indicating that this large linkage group has been conserved through 80 million years of evolution. The gene coding for mannose phosphate isomerase and pyruvate kivase - 3 have been assigned to human chromosome 15. In the mouse MPi is assigned to chromosome 9. Data derived from our studies assigns the gene coding for pyruvate kinase to mouse chromosome 9 these indicating a second conserved linkage group between man and mouse. Similar data has been obtained for the genes coding for glucosphosphate isomerase and peptidase-D which are assigned to chromosome 19 in man and chromosome 7 in the mouse.

In addition to the demonstration of conserved linkage groups between mouse and man, several new mouse gene assignments have resulted from these studies, namely; adenylate kinase - 1 to chromosome 2, peptidase - S to chromosome 5, lactate dehydrogenase - A and isocitrate dehydrogenase - 2 to chromosome 7, tripeptidase - 1 and hexokinase to chromosome 10; red cell acid phosphatase to chromosome 12; and dipeptidase - 2 to chromosome 18. Similar studies are now underway to map chinese hamster and rabbit genes. These studies increase our understanding of the evolution of the mammalian genome which is fundamental to the extrapolation of biological and biomedical data from lab animals to man.

3.11 NON-RANDOM SEGREGATION OF CHROMOSOMES IN HYBRID CELLS (Moss, Brown, Lalley, Minna)

The outstanding characteristic of interspecies somatic cell hybrids is the preferential segregation of one of the parental sets of chromosomes. The mechanism by which this occurs and is regulated is unknown. We have studied chromosome segration in hybrid cells formed from several different parental cell combinations (human and mouse; human and Chinese hamster; mouse and chinese hamster). Hybrids used in these studies were derived either from the fusion of cultured cells or from the fusion of cultured cells from one species with uncultivated, normal differentiated cells from another species. Previously it has been thought that chromosome segregation in somatic cell hybrids was a random event. However, our data indicate that this in not the case. The most extreme example encountered so far is in the Chinese hamster x mouse hybrids segregating mouse chromosomes. In thirty hybrid clones that were analysed cytogenetically by Dr. Uta Francke (UCSD) Chromosome 11 was absent from every hybrid clone analysed. No other chromosome was found to segregate in this manner. The explanation for the preferential elimination of chromosome 11 is not clear. However, it may be that this mouse chromosome

Carries information which is lethal or growth regulatory in this hybrid cell combination. On the other extreme, all Chinese hamster x mouse hybrids were found to contain mouse chromosome 15, frequently in multiple copies. Since trisomies of chromosome 15 have been reported in certain mouse leukemias, the malignant properties of these hybrids is being investigated. These data suggest mouse chromosome 15 may carry genes promoting cell survival or replication. The non-random segregation of human chromosomes in human and rodent cell hybrids has also been observed including hybrids formed with human tumor cells. The initial data indicate that the preferential loss or retention of specific human chromosomes may depend on the origin of the parental cell. We are specifically comparing chromosome segregation patterns in hybrids formed with normall human marrow cells with hybrids formed with human acute leukemia cells. We are asking if there are human chromsomes comparable to mouse chromosome 11 (growth controlling or lethal), and mouse chromosome 15 (growth promoting or highly retained) using the hybrid cell test system.

3.12 COMPUTER PROGRAMMING FOR GENE MAPPING (Moss)

Somatic cell hybridzation is a powerful technique for the assignment of gene linkage groups. Because of the great variety of cellular properties which can be determined and correlated with one another in these hybrid cells, it is a difficult and error-prone task to analyze these data. Thus, a computer data management system has been devised to record extensive data on the several hundred different cell lines used in our laboratory. This system utilizes a terminal linked to the NIH computer facility. Gene segregation analyses which would otherwise require several days can now be done accurately in minutes. Errors in the data which would else remain undetected are easily found and corrected. The ready access to detailed experimental results on specific cell lines provides a means by which the design of future experiments is facilitated by allowing the selection of cell lines with particular sought characteristics.

3.13 MAPPING OF HUMAN GENES RELATED TO CARCINOGEN METABOLISM USING HYBRID CELLS (Brown, Minna)

Aryl hydrocarbon hydroxylase (AHH) expression in human x mouse somatic cell hybrids. Microsomal mixed-function oxygenases are key factors in mammalian metabolism of xenobiotics. Genetic dissection of the regulatory mechanisms controlling the expression of these enzymes in man would greatly aid the understanding of the human disposition of toxic and carcinogenic chemicals. In collaboration with Dr. H. Gelboin's Lab (NCI) we have been attempting to correlate the expression of a mixed-function oxygenase, AHH, with the presence of human chromosomes using somatic cell hybrids. Hybrid cells were formed from mouse RAG cells deficient in AHH activity and fresh human bone marrow cells. AHH expression in 12 primary and 19 subclones correlated with the presence of human isozymes malate dehydrogenase and isocitrate dehydrogenase, known markers for human chromosome 2. Isozyme markers for 19 other chromosomes were shown to segregate independently of AHH expression. Thus, we can conclude that gene(s) on chromosome 2 are required for AHH expression in these hybrid clones. We have studied the relationship between constituative and induced AHH activity and found that constituative activity is also dependent on gene(s) present on human chromosome 2.

Studies are in progress to prove the activity being measured in the hybrids is human AHH using high pressure liquid chromatography. Using this technique, Dr. Gelboin's laboratory has found that a metabolic product of the AHH reaction benzo(a)pyrene 9,10 diol, is formed by mouse tissue but not by human tissues. Studies on the hybrid cells are in progress. It is planned to extend these studies to include an analysis of the several enzymes involved in the metabolism of carcinogenic aromatic hydrocarbons, specifically epoxide hydrase, using hybrid cells to determine the gene linkages.

3.14 IMMUNOTHERAPY USING SOMATIC CELL HYBRIDS (Carney, Minna)

We would like to use hybrid cells for immunotherapy in patients. We have developed methods for generating interspecies hybrids by fusing rodent cells directly to tumor cells taken from patients. These hybrids can replicate rapidly to give large numbers of cells and thus rodent x human tumor cell hybrids can be available for therapy a few months after primary surgical treatment. The hybrids present human antigens on a mosaic human - rodent background and thus potentially could present weak tumor specific antigens in a new format to the host. Because the tumor antigens may represent differentiation productss we are exploring the use of rodent cell lines differentiating along the same pathway as the human tumor cells in addition to using standard mouse and Chinese hamster parent cells.

We are collaborating with the Laboratory of Dr. H. Rapp in testing the hypothesis on the Guinea pig hepatoma system. This model has already shown several desirable features: namely the hepatoma metastasizes, can be treated with at least one form of non-specific immunotherapy, BCG, and the hepatomas by themselves do not induced clinically relevant immunity in the Guinea pig. Hybrids are being produced by fusing human, mouse or Chinese hamster cells with the Guinea pig hepatoma cells which will then be used to treat the animals after surgical debulking.

An interesting sidelight to this project is the identification of cell lines producing condition medium and growth factors for the hepatoma cells. The hepatoma cells in the ascitic form when placed into culture alone do not replicate. However, if they are co-cultured with several different cell lines including a human lung cancer line, they replicate well. This indicates the human lung cancer line is producing a growth factor required by the hepatoma cells. We are trying to partially characterize this phenomenon.

3.15 EFFECT OF PRIMATE TROPIC RNA VIRUSES ON HUMAN LUNG CANCER GROWTH IN VITRO AND IN VIVO (Carney, Gazdar).

We tested the effect of primate tropic RNA Type C viruses on the growth of lung cancer cells. A bronchiolalveolar cell line was infected with baboon M7 viruses and then clonal lines derived. These were compared for plating efficiency in liquid medium, soft agar, and for growth in the nude mouse with uninfected cells. We found no differences between the uninfected and the cells supporting the replication of M7 virus. The project is terminated.

3.16 GENE TRANSFER BY MEANS OF PHOSPHOLIPID VESICLES (LIPOCHROMOSOMES)

We have participated in experiments originating in the Laboratory of Drs. Mukherjee, and Schulman, NICHD concerning the use of phospholipid vesicles to transfer chromosomes. A human rodent hybrid cell containing only a fragment of the human X chromosome was used to prepare metaphase chromosomes and these were placed into phospholipid vesicles constructed of cholesterol and egg lecithin. Dr. Mukherjee, fused these vesicles with PEG to a hypoxanthine phosphoribosyl transferase (HPRT) deficient mouse cell and isolated cell lines in selective medium. Dr. Lalley of our laboratory tested homogenates of the hybrids for the expression of human X linked genes. He found that not only had human HPRT been transfered but also human G6PD and PGK enzyme activities. These results represent possibly the first time a large segement of chromosome has been incorporated in another cell by gene transfer techniques. By transferring the human genes into a species other than mouse we are trying to rule out the possibility of inadvertant transfer of hybrid cells with the metaphase chromosomes.

3.17 USSR-USA SCIENTIST EXCHANGE IN THE CANCER GENETICS FIELD

Dr.Eugenia Kakpakova visited our laboratory for 4 months as part of the USSR-USA exchange program in cancer genetics. During this period she tested hybrids between mouse 3T3 cells and Djungarian hamster tumor cells she had prepared in the USSR and brought to the USA. These were tested in nude mice and found to be tumorogenic. We are completing the isozyme analysis of these hybrids in the USA while Dr. Kakpakova is completing the chromosome analysis in the USSR. In a second set of experiments Dr. Kakpakova tried to make hybrids between normal Djungarian hamster cells and malignant human, mouse and Chinese hamster cells using PEG fusion. Unfortunately after many (16 different) attempts no hybrids were obtained. We are continuing to try to make hybrids with cells isolated from the Djungarian hamster she brought from the USSR with human malignant cells so that we can send these to her in Russia.

3.18 EFFECTS OF STREPTOZOTOCIN ON TYPE C VIRUS PRODUCTION IN MICE (Gazdar)

We have been studying the side effects of chemotherapeutic agents in model animal systems. Multiple injections of streptozotocin (SZ) in outbred Charles River (CD-1) mice produce diabetes characterized by pancreatic insulitis and progressive destruction of beta cells. Electron microscopic studies demonstrate type C virus particles in the beta cells of these mice, but not in those of untreated controls. Type C viruses are seen in the spleens of both treated and control mice. We have tested homogenates from treated and control mice for type C virus production. While ecotropic strains (N-tropic) of virus can readily be obtained from the spleen of all mice, virus can only be obtained from the pancreases of diabetogenic, SZ treated mice. These studies demonstrate than in vivo activation of type C viruses is possible, and that a chemotherapeutic agent currently in use in human patients may activate potentially oncogenic viruses in the target tissues.

3.19 LABORATORY STUDIES OF THE KINETICS OF HUMAN MALIGNANCIES AND MEGAKARYOPOIESIS (Dr. Bunn, Ford, Nugent, and Brigham)

These investigations are concentrated in two main areas; cellular kinetics of human malignancies and megakaryopoiesis.

Methodology of cell Kinetic Studies: We have demonstrated that commercially available instruments can accurately count grains and cells in biologic specimens. We have also collaborated with Dr. Shackney and Mr. Pendland in contracting Artec System to develop automated instrumentation for providing rapid grain counting and DNA content measurements. We have shown that rapid radioautography (6-24 hours) is dependent upon the dose of tritiated thymidine. The use of high specific activity of 3HTdR, scintillation fluid, or cold developing are far less important than the dose. Emulsion exposure times must be longer when low dose of 3HTdR are employed.

We have performed cytokinetic studies on 10 patients with mycosis fungoides and the Sezary syndrome in collaboration with Dr. Schackney. We have shown that the malignant cells proliferate in lymph nodes as well as in cutaneous sites, and that the cells proliferate slowly. These studies have defined the sites and source of proliferating cells and the kinetics of proliferation in the cutaneous T Cell lymphomas.

Malignant plasma cells in patients with multiple myeloma have low labelling indices and few cells with S phase DNA contents. Protein production is predominant in G1 (Go) cells. The growth fraction remains low even after response to drug therapy.

We have found labelling indices vary by as much as 50% between metastatic sites in the same patient with solid tumors such as small cell bronchogenic carincoma. Labelling indices obtained after in vitro incubation of needle aspirates with 3HTdR are similar to those obtained with in vivo labelling.

Megakaryopoiesis: Colcemid , a known stimulator of megakaryopoiesis, was not able to reduce the thrombcyotpenia induced by cyclophosphamide in a a mouse model system. We are currently evaluating an in vitro colony assay system for megakaryopoiesis in order to test patients during their course of chemotherapy.

3.20 LABORATORY STUDIES OF TUMOR BLOOD FLOW (Dr. Cohen)

Studies of blood flow to experimental murine solid tumors is continuing. As previously indicated, considerate stasis exists in the tumor circulation. We are currently attempting to reduce stasis experimentally by means such as decreasing whole blood viscosity and anticoagulation. Assays of tumor blood flow have been standardized over the past year and the model currently yield reproducible results. While these studies are simple in concept, they bear on one of the fundamental problems in treating chemotherapeutically resistant human tumors.

3.21 EARLY DETECTION OF INFECTION USING IMMUNOASSAYS (Dr. Fossieck, Fedorko)

As therapy becomes more intensive in solid tumor patients the need for early diagnosis of infection and evaluation of unexplained fevers increases. The following experiments provide new techniques for dealing with these clinical problems. Using a system previously developed in this lab in collaboration with Dr. R. Parker of the Infectious Disease Section, WVAH, counterimmunoelectrophoresis (CIE) was used to rapidly identify blood culture isolates in 75 cases of S. pneumoniae, 15 cases of K. penuemoniae, 2 cases of H. influenzae, and 1 case of N. meningiditis bacteremia. Success was achieved in over 90% of cases and cross reactivity in 150 cases of bacteremia due to other bacteria was less than 1%.

Blood cultures inoculated with bacteria are being studied with Gram stain, CIE, and quantitative cultures to determine the temporal relationships, number of colony forming units necessary to produce a positive CIE, and utility of the procedure.

A new CIE for C. albicans was developed. Patients in the NCI-VA Oncology program are being followed prospectively with CIE surveillance for development of infection with S. aureus and C. albicans. To date 378 initial or sequential studies have been performed on 70 patients.

Animal models are being developed for the study of CIE in experimental staphylococcal and candida infections.

3.22 STUDIES OF THE EFFECT OF HYPERBARIC OXYGEN ON CHEMOTHERAPY OF TRANSPLANTABLE MURINE TUMORS (Dr. Ihde)

Administration of radiotherapy in hyperbaric oxygen increases the radiosensitivity of certain murine tumors. Previous investigations have revealed that administration of hyperbaric oxygen at sufficient pressures (up to 4 ATA) and for sufficient time periods (up to 4 hrs) will result in suppression of 3H-TdR labelling index (LI) and incorporation into DNA in several subcutaneous and intraperitoneal murine tumors studied in vivo.

When SQ B16 melanoma in C57Bl/6 mice was studied after only 15 min of hyperbaric oxygen at 1-3 ATA, however, both 3H-TdR LI and incorporation into DNA increased. This was not true for the three other tumor systems which were investigated. It was therefore elected to invetsigate the effect of varying doses of cyclophosphamide given to normal mice and those bearing the previously studied transplantable tumors in air and after exposure to 15 min or 4 hr of hyperbaric oxygen at two different pressures. These studies are in progress.

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01-CM-06810-13 PO

PERIOD COVERED
July 1, 1976 to Sept. 30, 1977

TITLE OF PROJECT (80 characters or less)
Study and Treatment of Cancer in Children and Young Adults

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
35	20	15

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS X☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Acute leukemia, Non-Hodgkin's lymphomas (e.g., Burkitt's), neuroblastoma, rhabdomyosarcoma, osteosarcoma, Ewing's sarcoma and aplastic anemia are studied. In leukemia, we have devised therapeutic regimens which may allow maximum tumor cell kill while minimizing sequellae. Emphasis is on tailoring treatment to individual prognostic variables, and improving the therapeutic ratio in CNS prophylaxis. In the solid tumors, we have developed combined modality approaches to primary disease. In refractory tumors, we are studying the utility of high-dose-therapy and the role of supportive care (platelet and white cell transfusion, laminar-flow protection, autologous bone marrow rescue, total parenteral nutrition) in permitting such therapy. Phase I trials are also conducted in refractory tumors; agents include maytansine and poly I:C/poly-l-lysine. Studies of glutaminase, AMSA, and coformycin are starting. Aplastic anemia is studied as a model for hematopoietic differentiation. We are exploring the biology of selected tumors, including kinetics, immunology, virology, tumor markers, genetics, biochemistry, and pharmacology. Also studied are the effects of cancer and its treatment on growth, development, and organ function. The psychosocial concomitants of cancer are explored.

Objectives:

1) To devise effective therapeutic regimens in acute leukemia which allow maximum tumor cell kill with drugs and radiotherapy, while leaving intact effective elements of the immune response and minimizing therapeutic sequellae--especially in the CNS.

2) To develop effective combined modality treatment approaches to previously untreated childhood solid tumors, including rhabdomyosarcoma, neuroblastoma, non-Hodgkin's lymphoma, osteogenic sarcoma, and Ewing's sarcoma.

3) To study the possible role of high-dose ("ablative") chemotherapy in advanced leukemia and solid tumors, and the role of supportive care (platelet and white cell transfusion, laminar-flow protection, bone marrow rescue, hyperalimentation) in permitting such ablative therapy.

4) To study the biology of selected tumors, including kinetics, immunology, virology, prognostic markers, karyotypes, biochemistry, and pharmacology.

5) To assess the pharmacology of selected atents, e.g., high-dose methotrexate, AMSA, maytansine, poly I:C/poly-l-lysine, glutaminase, and coformycin in humans.

6) To study the short- and long-term effects of malignant disease and cancer treatment on growth, development, and organ function.

7) To study the psychosocial concomitants of cancer in young patients, their families, and their therapists.

Methods and Major Findings:

A. Therapeutic Regimens for Acute Lymphatic Leukemia (ALL)

1. Protocol 72-1 was designed to compare the utility of maintenance chemotherapy versus maintenance chemotherapy plus immunotherapy. All patients received 5 day remission induction courses of POMP (prednisone, vincristine, methotrexate, 6-MP) until an M1 marrow was achieved. All patients were then treated with systemic consolidation chemotherapy and CNS prophylaxis. The latter treatment consisted of 2400 R to the brain and intrathecal cytosine arabinoside or methotrexate given during irradiation and monthly thereafter for 30 months. Following completion of consolidation chemotherapy and cranial irradiation, patients began a 34 month course of maintenance therapy, divided into 6 treatment blocks. Each block was composed of a 4 month (Phase I) and a 2 month (Phase II) cycle. All patients received identical maintenance therapy during Phase I consisting of 6-MP, methotrexate, vincristine and prednisone. Patients were randomized to receive during Phase II one of three different regimens: 1 of 2 methotrexate schedules, or BCG plus allogeneic cells. A total of 70 patients were entered on this study which was closed to further

entry in 1975. Sixty-three patients (90%) achieved complete remission. Of these patients, 29 (46%) successfully completed chemotherapy and have been off of all treatment for up to three years. Twenty-four patients (39%) are in complete remission at 6 years. Five patients relapsed while unmaintained. When one considers the particular set of apparent prognostic variables within our patient population (age, initial white count) these results are very similar to those obtained in other major centers during this period of time. However, we found no significant difference in either the relapse rate or the duration of remission between patients treated with maintenance chemotherapy alone or maintenance chemotherapy plus immunotherapy.

2. Protocol 75-1 - Retrospective analysis of protocol 72-1 demonstrated that patients could be divided into good and poor prognostic groups on the basis of certain factors. Poor prognosis was associated with failure to achieve remission following four courses of POMP induction therapy; the presence of meningeal leukemia at the time of the first CSF sampling; age; and initial WBC count exceeding 50,000/mm^3. Of patients in the good prognosis category, 80% remained in remission at 40 months. In the 75-1 study, patients were segregated on the basis of the previously identified prognostic factors. Patients in the good prognosis group were treated conservatively as in the 72-1 protocol. Patients in the poor prognostic group were treated with a much more intensive chemotherapeutic regimen and randomized to receive or not receive immunotherapy during induction and maintenance. All poor prognosis patients received four courses of POMP induction followed by early intensification with L-asparaginase and cytoxan. After CNS prophylaxis, maintenance therapy was offered consisting of methotrexate, 6-MP, vincristine, and prednisone as well as high-dose cytoxan. All patients in the poor prognosis group also received a full-course of cytosine arabinoside and duanomycin as a late intensification regimen 6 months following the achievement of remission. Patients randomized to receive immunotherapy were given the methanol-extraction-residue of BCG (MER). Twenty patients with ALL have been entered on the 75-1 protocol, four in the good prognosis group and 16 in the poor prognosis group. (This distribution appears to reflect a trend in the NIH referral pattern). Nineteen of the 20 patients achieved complete remission; of the 16 poor prognosis patients, 6 received MER and 10 were randomized not to receive MER. Seven of the 16 patients in the poor prognosis group have already relapsed, with no significant difference between those receiving MER and those not receiving immunotherapy. All four patients in the good prognosis group remain in complete remission. Projected remission duration and survival data in the poor prognosis group has indicated a relapse rate and remission duration for the poor prognosis patients no different than was observed for the earlier 72-1 poor prognosis patients. Thus, intensification of chemotherapy as practiced in protocol 75-1 does not improve the outlook for poor prognosis ALL patients. We have therefore closed this study to further entry and have initiated a new protocol for the treatment of all patients with ALL (77-02).

3. Protocol 77-02 - In this study, all patients with ALL receive identical systemic treatment without regard to presumed prognostic variables. Our hope is to learn more about the unique biology of each patient such that specific mechanisms of response or failure can be explicated. This information will then be used to guide further treatment. Additionally, this protocol examines prophylactic treatment of the CNS. As indicated previously, the classic therapy offered during CNS prophylaxis consists of cranial irradiation plus intrathecal chemotherapy. We have noted an unexpectedly high incidence (more than 50%) of important neurologic sequellae consequent upon this treatment, and protocol 77-02 attempts to learn in a controlled study whether systemic chemotherapy constructed with regard to CNS pharmacokinetics will produce as good or better a therapeutic result as intrathecal chemtherapy plus cranial irradiation. Our hope is that the former regimen, while avoiding cranial irradiation, will be associated with fewer significant neurologic sequellae.

B. Studies on CNS Leukemia

1. Intrathecal Methotrexate vs. Cytosine Arabinoside
On protocol 72-1, patients were randomized to receive intrathecal cytosine arabinoside or methotrexate as a component of CNS prophylaxis. One of 32 patients receiving methotrexate and 3 of 29 receiving cytosine arabinoside have developed meningeal leukemia. While these data suggest that both drugs are equally useful as the intrathecal components of CNS prophylaxis, we found that 2 patients that received cytosine arabinoside became blind. While there is no proof of the association between cytosine and blindness, we believe that methotrexate remains the treatment of choice.

2. Treatment of overt meningeal leukemia - Protocol 73-6
This protocol for the treatment of established meningeal leukemia was closed to further entry in 1976. This study was designed to answer the question whether small frequent doses of intraventricular methotrexate would be more effective and less toxic than the conventional bolus dose of this drug. All patients underwent surgical implantation of a subcutaneous Ommaya reservoir and were randomized to receive via this reservoir either a conventional methotrexate dose of 12 mg/mm^2 twice weekly, or 1 mg/mm^2 every 12 hrs for 3 days. Eighteen of 19 patients entered in the study achieved complete remission of meningeal leukemia. Two of 9 patients in the low-dose group have developed relapse of their CNS leukemia, and 1 of 9 in the conventional dosage group. Median remission duration for the entire study is 916 + days. Thus there was no significant difference between the two treatment groups in relapse rate or duration of CNS remission. However, significant neurotoxicity occurred in 7 out of 10 patients in the conventional dose group but only 1 of 9 patients in the low-dose group. These data suggest that the frequent low-dose methotrexate schedule

(made practical through the use of the Ommaya reservoir) is as effective against overt meningeal leukemia as is the conventional methotrexate schedule, but with a much lower risk of neurotoxicity.

3. Intrathecal versus intraventricular methotrexate in overt meningeal leukemia. We compared intraventricular methotrexate delivered via the Ommaya reservoir versus intrathecal methotrexate in the treatment of established meningeal leukemia using each patient as his own control. Ten children who had recurrence of CNS leukemia despite monthly intrathecal methotrexate were reinduced and maintained with intraventricular methotrexate. Remission duration with intraventricular methotrexate was compared with the patient's previous remission duration in association with intrathecal methotrexate. Seven patients had a longer and one a shorter CNS remission with intraventricular methotrexate than with intrathecal methotrexate. Three patients who died of systemic leukemia while remaining in CNS remission on intraventricular methotrexate had no evidence of CNS leukemia at autopsy. There were three CNS relapses during 4,009 patient days at risk in the intraventricular treatment group in contrast to 10 CNS relapses in 3,312 days at risk in the intrathecal treatment group. These data suggest that intraventricular chemotherapy is more effective against overt CNS leukemia than intrathecal chemotherapy.

4. Sequellae of prophylaxis and treatment of meningeal leukemia: Utility of computer-assisted tomography in detecting methotrexate leukoencephalopathy after treatment of overt CNS leukemia

Although methotrexate has been effective in treating overt meningeal leukemia, its administration can result in significant toxicity, including the recently recognized syndrome of methotrexate leukoencephalopathy (confusion, forgetfulness, poor mentation, and/or progressive dementia). Histopathological examination of brain tissue from these patients has revealed multiple foci of necrosis, mainly involving the white matter. We studied five children with acute lymphoblastic leukemia who had developed encephalopathy following treatment with intrathecal or intraventricular methotrexate by use of computed tomographic scanning. Utilizing this new diagnostic technique, we were able to define two types of CT scan abnormality in these patients: A symmetrically decreased attenuation coefficient in the centrum ovale of each hemisphere and in the region surrounding the lateral ventricles, and, in some patients, calcifications in the basal ganglia. The apparent ability of CT scanning to detect methotrexate leukencephalopathy suggests that both baseline and follow-up CT scans should be performed in all patients so treated.

5. Abnormal computed tomography scans in children with acute lymphoblastic leukemia following central nervous system prophylaxis.

Prompted by our observations on the utility of the CT scan to detect central nervous system lesions associated with methotrexate leukoencephalopathy, we instituted a study of children with ALL who had been given central nervous system prophylaxis. Thirty-seven patients with ALL who had received 2400 r of cranial irradiation and either intrathecal methotrexate or cytosine arabinoside (protocol 72-1) were recalled for CT scanning 20-67 months following the initiation of prophylaxis. All patients were asymptomatic at the time of study. Seventeen out of 32 patients (53%) had one or more abnormal CT scan findings (ventricular dilatation, subarachnoid space dilatation, decreased attenuation coefficient in white matter, and/or intracerebral calcifications). The only abnormality found in the 18 patients who had received cytosine arabinoside was ventricular and subarachnoid space dilatation (9 patients). However, of the 14 patients in the methotrexate group, 8 patients demonstrated ventricular dilatation, subarachnoid space dilatation, decreased attenuation coefficient, and intracerebral calcification. Both of the latter findings have been associated with methotrexate leukoencephalopathy (see previous paragraph). The presence of these dramatic but unanticipated CT scan findings in patients who had received central nervous system prophylaxis is of obvious concern, and has prompted us to initiate extensive preclinical and clinical investigation of possible alternative CNS treatments (e.g., 42 hour IV methotrexate infusion without cranial irradiation).

C. Prognostic Assays in ALL

1. Adenosine deaminase - to facilitate the selection of poor prognosis patients for tailored regimens, it is important that new markers be developed to distinguish functional subcategories of lymphoblasts. In addition to a continuing examination of the role of immunological cell surface markers, we are investigating possible biochemical means of distinguishing lymphoblast subtypes. In this regard, we have found that the levels of adenosine deaminase are significantly elevated in lymphoblasts as compared to the levels in normal lymphocytes. Moreover, we have found a significant correlation between these elevated adenosine deaminase levels and lymphoblasts with "T-cell" markers, whereas adenosine deaminase levels are not increased in "null" cell leukemia. This finding may have diagnostic, prognostic, and therapeutic implications.

2. In view of the known difference in susceptibility to glucocorticoids among functional subcategories of lymphocytes in the mouse, and the awareness that different subcategories of acute lymphocytic leukemia (ALL) may represent proliferation of different clones of lymphoblasts, we examined lymphoblast populations with known surface markers for differences in specific glucocorticoid receptor (GR) activity. Lymphoblasts were divided into those which formed sheep erythrocyte rosettes ("T" lymphoblasts) and those which lacked all surface markers ("null" lymphoblasts). The latter were further subdivided by their ability to stimulate allogeneic donors in mixed lymphocyte culture (MLC). Lack of stimulation in MLC is a characteristic of "T" cells. Eighteen

patients with T lymphoblasts had binding sites ranging from 0 - 5,887 sites/cell (median 2,173) ($p < .001$). Nine patients whose lymphoblasts lacked identifiable surface markers but failed to stimulate in MLC had intermediate values for receptor sites. Thus it appears that "null" lymphoblasts are more likely to have higher numbers of GR than "T" lymphoblasts. The differential capacity of lymphoblasts to bind steroid may prove useful in designing therapeutic regimens for the different subcategories of ALL.

D. Treatment of Refractory Acute Leukemia with High-Dose Chemotherapy - A significant number of patients with acute leukemia continue to experience relapse and rapidly develop resistance to conventional chemotherapy. High-dose chemotherapy followed by allogeneic bone marrow transplantation has been attempted in this circumstance and has met with a degree of success. The question arises whether the high-dose chemotherapy alone can induce a significant percentage of remissions. In this study, we employed a high-dose chemotherapy regimen in relapse leukemia patients. The regimen was similar to one previously used as preparation for bone marrow transplantation. Seventeen patients (12 ALL and 5 AML) with one or more previous relapses were treated with "TEAM" (cytoxan 50 mg/kg/ day, days 1, 2, and 6; Ara-C, 100 mg/M^2 days 3-6; 6-thioguine, 100 mg/M^2, days 3-6; and methyl CCNU, 150 mg/M^2, day 5). Of the 17 treated patients, only three attained complete remission, the longest lasting for 73 days. The results were disappointing and toxicity was great. Thus, we find little to support the use of this high-dose combination chemotherapy regimen in refractory acute leukemia.

E. Studies on the Treatment of Solid Tumors

1. Utility of High-dose Chemotherapy in Metastatic Neuroblastoma - Protocol 75-8 seeks to determine whether very high doses of established agents will be useful in the treatment of metastatic neuroblastoma, a disease with a current 2 year survival of less than 5%. The study employs a combination of those agents heretofore proven most effective against neuroblastoma, i.e., vincristine, DTIC, and cyclophosphamide. The cyclophosphamide is administered in progressively higher doses, in the hope that the tumor will demonstrate no plateau in its dose-response curve. Vigorous hematologic and antimicrobial supportive care are employed in tandem with this protocol in order to counter the profound myelosuppressive effects of the escalating cyclophosphamide. Thus far 6 patients have been enrolled in the protocol. Four patients completed three full cycles of chemotherapy; 2 completed only 2 cycles. Although all 6 patients demonstrated partial clinical responses, all died of causes directly or indirectly related to the chemotherapy *per se* (1 patient died in hepatic failure, presumably chemotherapy-induced, and the remaining five died of infections which occurred during the period of bone marrow hypoplasia following chemotherapy). Autopsies were performed in 5 of the 6 patients. One patient who

had completed 3 courses of chemotherapy had no evidence of disease at post mortem examination; however the remaining 4 patients autopsied all had detectable disease. Four more patients will be enrolled in the study which will then be terminated if results do not improve.

2. Studies on the Treatment of Metastatic Osteogenic sarcoma
   a. Protocol 75-3 - Much progress has been made in the management of primary osteogenic sarcoma; surgical resection of the tumor plus high-dose adjuvant chemotherapy has produced a 4 year survival rate of approximately 50% among patients initially free of overt metastases. Once overt metastases have appeared (usually pulmonary), the prognosis remains very poor. In protocol 75-3 we explored the use of very intensive combination chemotherapy for patients either presenting with pulmonary metastases at diagnosis or developing overt metastases while on adjuvant chemotherapy. Eleven patients with metastatic osteogenic sarcoma were treated with sequential combination chemotherapy consisting of cyclophosphamide, adriamycin, and, in some cases, high-dose methotrexate. In the event of metastatic progression, the dose of cyclophosphamide was escalated sequentially by increasing the number of consecutive daily infusions of this drug (dose = 45 mg/kg/day). The results indicate that metastatic osteogenic sarcoma is marginally responsive to adriamycin and cyclophosphamide but rarely responsive to high-dose methotrexate as administered in this trial. The response of metastatic lesions was not improved by escalating the dose of cyclophosphamide. Methotrexate and adriamycin were well-tolerated, as were treatment cycles containing one day of cyclophosphamide. Cycles which included 2 or 3 consecutive days of cyclophosphamide were associated with severe but manageable toxicity. Cycles containing 4 consecutive days of cyclophosphamide caused prolonged but not irreversible pancytopenia.

   b. Protocol 77-03 - Because these results were disappointing, protocol 75-3 was terminated. A new study has been initiated in 1977 which emphasizes total surgical debulking of overt metastatic disease followed by intensive sequential treatment with all agents known to be active in this disease. Following aggressive pulmonary resection, patients are given 1 week of high-dose methotrexate followed by exposure to 3 alkylating agents (L-PAM, DTIC, and cyclophosphamide). During the severe bone marrow hypoplasia which follows such treatment, patients are offered laminar-air-flow isolation, platelet and granulocyte transfusions, and autologous bone marrow rescue where appropriate. Following recovery of peripheral counts, patients are then exposed to 1 week of treatment with adriamycin and cis-platinum. Eighteen months of maintenance with high-dose methotrexate alternating with adriamycin then follow. Patients who cannot be totally debulked at the initiation of this study are treated with high-dose methotrexate in the hope that their lesions will be altered in such a way as to then permit total surgical resection, followed by the intensive sequential chemotherapy described above. To date, six patients have been enrolled in this new study with no recurrence of overt metastases but a mean follow-up time of less than six months.

3. Rhabdomyosarcoma

a. Protocol 76-4 (Primary treatment) - Previous studies have shown that combination chemotherapy has significantly improved the survival of children with localized rhabdomyosarcoma. Initial therapy for these patients has usually included surgical excision. However, the sites of tumor involvement (head and neck, GU) often make such surgery mutilative or functionally impairing. Part A of this protocol is designed to assess the utility of initial chemotherapy in "pharmacologically debulking" the primary tumor so as to provide an alternative to "radical surgery." Patients with lesions judged to be surgically resectable are randomized to either initial chemotherapy (followed by radiation and surgery) or to the standard approach of surgery, followed by chemotherapy and radiotherapy. In contrast to the situation with localized tumors, combination chemotherapy has failed to improve the survival of patients with metastatic rhabdomyosarcoma. For such patients, part B of this protocol addresses the utility of intensive (escalating) combination chemotherapy (in conjunction with hematologic and microbiologic support) versus conventional dose combination chemotherapy. Six patients have entered this protocol (Part A or B) to date. One patient presented with potentially resectable, localized disease and was randomized to the "initial surgery" arm of Part A. Five patients presented with unresectable or metastatic disease. Three patients with metastases were randomized (Part B) to receive combination chemotherapy with conventional doses of vincristine, actinomycin, adriamycin, and cyclophosphamide. Radiation therapy was also administered to primary and metastatic sites. All achieved complete remission, but two patients relapsed six months later. The fourth Part B patient with metastases was randomized to the "intensive chemotherapy" arm, and although this patient's tumor responded poorly to the initial lower doses of chemotherapy, it showed an improved response to escalated doses of chemotherapy. However, toxicity was severe and the patient died with cyclophosphamide-induced hemorrhagic myocarditis. The fifth Part B patient presented with an unresectable rhabdomyosarcoma. Although a partial response was obtained with chemotherapy and radiation, the patient's disease recurred and was then unresponsive to chemotherapy.

b. Protocol 75-5 (relapse treatment)

Eight relapsed patients have entered this protocol which employs escalating cycles of intensive combination chemotherapy. Four patients completed the protocol, one attaining a complete remissi and three others partial remission or stabilization. All four patients, however, subsequently relapsed. Four other patients died while on study. Two of these patients had shown significant tumor regression but experienced fatal toxicity (systemic candidiasis and radiation-induced pericarditis). The remaining two patients showed initial tumor stabilization but later died of progressive disease.

c. Diagnostic Studies -

Pathological specimens from patients with undifferentiated sarcom or rhabdomyosarcoma often present a diagnostic dilemma. To clari this situation, electron microscopy and immunofluorescent labelin with antimuscle antibody are being utilized. In addition, tumor specimens from two patients have been placed into tissue culture should provide a useful resource for studies on differentiation, kinetics, and metabolism. Previous studies have shown that tumor from patients with rhabdomyosarcoma have abnormalities in myosin banding; these studies are continuing, a major objective being the elucidation of potential tumor markers. Selected patients are studied with radio-autography and flow-microfluorimetry to assess the kinetics of this tumor.

4. Studies on the Treatment of Burkitt's Lymphoma

a. Primary treatment -

Clinical studies in Burkitt's lymphoma have been directed towards continued study of the combination therapy (COMP) consisting of cyclophosphamide, vincristine, methotrexate and prednisone commenced in June 1975. Two courses of this therapy are given followed by two doses of cyclophosphamide (each 45 mg/kg) and abdominal irradiation to patients with abdominal tumor. Twenty-one patient have been entered into this study. All except 2 had abdominal tumor and in 3 all abdominal tumor was resected. As anticipated the 5 patients commencing therapy without abdominal tumor have do well with no relapses so far (follow-up 3-21 months). Of the remaining patients, 8 of 16 remain in remission (3-17 months) and 8 have relapsed. However, of the relapsing patients, 2 had only nervous system disease (meningeal and cranial nerve involvement) relapse and both responded completely to intrathecal ara-C/MTX p cranial irradiation and additional systemic therapy. These two patients have been in remission for 5 and 6 months so far. One patient achieved reinduction with COMP therapy alone and 2 others were treated with BACT therapy (see below) and have been in complete remission for 9 and 19 months. Two patients died from tumor resistant to chemotherapy and the eighth died during reinduction from gram-negative sepsis. Further follow-up is clearly needed before the results in this group of patients can be fully assessed, but at this point the overall continuous disease-free rate (i.e., since first induction) is 13/21 or 62% while the current disease-free rate (including long term remissions post-relapse) is 18/21 or 86%. Since only 3 patients have been in first remission for less than 6 months, these results are unlikely to change dramatically. It is of interest that although the relapse rate does not differ significantly from that of patients treated on the previous protocol (cytoxan, methotrexate and vincristine for three courses), 50% of patients relapsing on COMP have achieved substantial second remissions of 5, 6, 9, and 21 months, and therefore the overall results appear to be considerably better. The reason for this is not clear.

Prolonged Complete Remission Following High-Dose Chemotherapy of Burkitt's Lymphoma in Relapse -
Fourteen patients with American Burkitt's lymphoma resistant to conventional chemotherapy were treated with high-dose combination chemotherapy (BACT: BCNU, Ara-C, 6-TG, and cyclophosphamide) and intensive supportive care. Four patients died shortly after chemotherapy, three of an acute carditis. All ten remaining patients demonstrated tumor regression and three remain in complete unmaintained remission 28+, 18+, and 8+ months after treatment. It is intriguing that all three of the long-term responders received autologous marrow infusions (see next section). These findings demonstrate that high-dose chemotherapy will benefit some patients with Burkitt's lymphoma unresponsive to conventional chemotherapy, but medullary and extramedullary toxicity of this treatment strategy remains a formidable obstacle.

Successful Engraftment of Cryopreserved Autologous Bone Marrow in Patients with Malignant Lymphoma -
In an effort to evaluate the possible utility of cryopreserved autologous bone marrow infusion in man, 19 patients with malignant lymphoma resistant to conventional chemotherapy were treated consecutively with high-dose chemotherapy followed in the first 12 patients by an infusion of their cryopreserved autologous bone marrow; the next 7 patients received chemotherapy alone and serve as controls. Following chemotherapy, severe leukopenia (<100 leukocytes/mm^3) lasted 6-10 (median = 9) days in patients receiving cryopreserved marrow compared to 10-29 (median = 18) days in controls ($p<.001$). Recovery to 1000 leukocytes/mm^3 occurred 10-18 (median = 13) days after chemotherapy in autograft recipients but was delayed until 12-38 (median = 23) days after chemotherapy in controls ($p<.001$). Autografted patients also recovered granulocyte and platelet function more rapidly than did controls. Cryopreserved autologous bone marrow infusions can hasten hemopoietic recovery in man after high-dose chemotherapy and this earlier reconstitution may be of clinical benefit to the patient.

d. Other Studies

Two patients with hypercalcemia were studied recently. These are the first examples of documented hypercalcemia in Burkitt's lymphoma. During the hypercalcemia phase there were very low levels of parathyroid hormone (PTH), indicating a non-PTH mediated mechanism. Both patients had multiple osteolytic lesions. Laboratory studies of cell lines derived from American Burkitt's lymphoma are described in report number NCI-06850-07.

5. Studies on the Treatment of Ewing's Sarcoma

a. Background -

In October of 1976, the patients on the Ewing's sarcoma study were transferred from the Radiation Oncology Branch to the Pediatric Oncology Branch. A total of approximately 125 patients had been entered on the Radiation Oncology Branch studies beginning in 1964 until the transition time. Of these, approximately 58 remained as active patients. In addition to radiation of the primary tumor, the patients had been treated with a variety of adjuvant chemotherapy regimens beginning with cyclophosphamide alone in 1964, vincristine and cyclophosphamide from 1968 through 1969, and with cyclophosphamide, vincristine and actinomycin D from 1969 through 1971. In 1971, what is basically the present protocol of the Radiation Oncology Branch was begun, consisting of radiation to the primary lesion followed by combination chemotherapy with vincristine, cyclophosphamide and adriamycin as well as CNS prophylaxis (2000 rads of whole brain irradiation and a single dose of intrathecal methotrexate). In mid-1974, a randomized trial of immunotherapy utilizing BCG was added to the current radiation-chemotherapy study, in conjunction with serial immunological evaluation of the patients using both *in vitro* and *in vivo* techniques. Immunological findings in these patients, particularly those obtained prior to the initiation of therapy, have been of considerable interest. The clinical results, however, are not significantly different from those obtained without the use of immunotherapy. In reviewing the overall experience with the patient population treated at the National Cancer Institute since 1964, as well as other data, problem areas in the treatment of Ewing's sarcoma became clearer. The prognosis of patients presenting with the diagnosis of Ewing's sarcoma depends on the site of the primary lesion and on the presence or absence of metastatic disease. Patients presenting with metastatic disease, irrespective of the site of the primary, have an extremely poor prognosis, with a median survival of approximately 22 months. The long-term survival rate is nil. Patients presenting without metastatic disease have a prognosis that depends on the site of the primary tumor. Primary lesions of the central axis carry the poorest outlook, and the prognosis becomes increasingly optimistic the further the primary lesion is from the central axis. Patients treated in the Radiation Oncology Branch with central axis lesions had approximately 54% disease-free survival at 18 months. Patients with more distal lesions had approximately 70% disease-free survival at 2 years.

b. New Studies -
With the background outlined above and with the availability of supportive care facilities in the Pediatric Oncology Branch, a series of studies have been initiated to evaluate the efficacy of intensive multimodality therapy in this disease. Patients presenting with metastatic disease are treated with high-dose radiation therapy (5000 rads) to the primary lesion and to known metastatic sites. Patients are also exposed to known effective chemotherapeutic agents and to total body irradiation, the efficacy of which was suggested in studies published approximately 10 years ago (TBI was not used then in conjunction with chemotherapy). Because of the high-dose chemotherapy employed, together with total body irradiation, patients who had no disease detectable in the marrow at the time of presentation have their marrow stored and reinfused subsequent to the high-dose chemotherapy. Immune competence as well as the kinetics of marrow reconstitution are being studied in these patients.

Patients who present with central axis lesions, but without evidence of metastatic disease, have been treated under the existing Radiation Oncology Branch protocol to the present time. As noted above, a more aggressive approach to these patients is warranted; we have now begun to treat them with a regimen similar to that being used for patients presenting with metastatic disease. Patients with distal lesions and without metastases are treated with radiation therapy to the primary lesion as well as vincristine, cytoxan, actinomycin D and adriamycin. They also receive prophylactic pulmonary irradiation based on preliminary data from the Intergroup Ewing's Study and the known efficacy of 1500 to 2000 rads whole-lung irradiation in controlling the common complication of pulmonary metastases. Patients who have recurrent disease and who have not been previously exposed to high-dose chemotherapy are now being given such treatment. Patients who have previously received high-dose chemotherapy go on to phase II studies. Since October of 1976, 8 new patients have been entered on these new protocols--two with metastatic disease, three with central axis primary lesions, and three with more distal primary lesions.

F. Interferon Inducers as Anti-tumor Agents
Polyriboinosinic-polyribocytidylic acid (poly I:poly C), an interferon inducer, was administered in multiple doses of 0.3-75 mg/m^2 to 26 patients with a variety of solid tumors, 9 with acute leukemia, and 2 with chronic myelogenous leukemia in blast crisis. Forty-four separate drug trials were comprised of various schedules and routes of administration. Toxic reactions included fever (in 66% of the trials), transient elevation of serum glutamic-oxaloacetic transaminase and serum glutamic-pyruvic transaminase (25%), minimal laboratory evidence of coagulation abnormalities (59%), and hypersensitivity (5%). These toxic manifestations did not

relate to dose level or magnitude of interferon induction. Poly I-poly C administered IV induced low serum concentrations of interferon in 24/38 trials (63%), but the correlation between drug dose and peak interferon titer was not linear. Poly I-poly C administered IV or IM was not effective as an inducer of interferon in the cerebrospinal fluid. Similarly, poly I-poly C administered IM or by inhalation did not produce detectable serum levels of inteferon. No patients experienced an objective tumor response to the administration of poly I-poly C, and most (76%) had progression of their disease while receiving the drug. A new phase I-II study was initiated in early 1976 employing poly I-Poly C/poly-l-lysine, a nuclease-resistant complex which has proven to be a more effective interferon inducer in man. Twenty patients have been entered on this study to date, the majority of whom have displayed an excellent interferon response to this inducer. No tumor response has yet been seen but the maximum tolerated dose has not been achieved.

G. Total Parenteral Nutrition Study -
Patients with metastatic rhabdomyosarcoma, neuroblastoma, Ewing's sarcoma, or osteosarcoma who are receiving intensive combination chemotherapy are randomized to receive (or not receive) intravenous hyperalimentation. Eight patients have been entered on this protocol, four in each arm. Careful metabolic and immunologic studies are being carried out in all patients. It is notable that even though the patients have been granulocytopenic (PMN $< 500/mm^3$) from 12 to 21 days, no catheter-related infections have occurred in the patients undergoing hyperalimentation. The relationship of nutritional integrity to host defenses, chemotherapeutic responsiveness, and drug toxicity may be of great importance in patients receiving very intensive drug regimens. This study should provide valuable insights into the metabolic changes which accompany intensive chemotherapy, as well as the utility of intravenous hyperalimentation in their prevention.

H. Psycho-social Concomitants of Cancer
We have previously reported on the families of patients who have life-threatening illness and on physicians who care for such patients. In particular, we explored the factors in the doctor-patient relation which promote anxiety in the doctor to the end that he is less effective than he might be. During the past year, our annual seminar for Clinical Associates on the Doctor-Patient Relation has continued with Dr. Kenneth Artiss as the seminar leader. The seminar continues to be highly successful and has proven to be a model for many other institutions throughout the country, including cancer centers and general hospitals. We have also initiated therapy groups for the families of ill children and for ill adolescents; these groups are conducted by oncologists and social workers who have received appropriate training in the principles of social and preventive psychiatry. We have also continued a collaborative research effort with the Laboratory of Developmental Psychology, NIMH (M. Yarrow, Chief). The objective of this research is to analyze developmental stages in children who have chronic and/or life-threatening disease, to learn the factors which promote normal or abnormal development, and the manipulations which may be of value in dealing with developmental abnormalities. This

collaboration has been strengthened during the past year by the addition of several new post-doctoral fellows in Dr. Yarrow's laboratory. In this regard, we have initiated new studies on the nature of communication between staff, family and patient; the effects of isolation within the laminar-flow room as a function of the age of the child; and the effects on mentation in patients who have received prophylactic irradiation of the central nervous system (as noted elsewhere, we have detected a significant drop in I.Q. among patients so treated).

I. Educational Programs -
Our extensive seminar, lecture and rounding schedule for associates and post-doctoral fellows has been continued. Two weekly Branch seminars emphasize disease entities in hematology-oncology, and research in basic sciences related to cancer. These seminars now attract a large audience from throughout the Institutes. The medical student elective program continues to be successful, with a significant proportion of students applying for eventual Clinical Associate positions.

Significance to Biomedical Research and the Program of the Institute:
Significant progress has been made in delineating the cell of origin in acute leukemia and in identifying factors which may be useful in predicting response to therapy. By dividing the acute leukemias vis-â-vis these prognostic factors, we hope to individualize therapy to the end that remission rate and duration will further improve. The study of steroid receptors is being pursued in the hope that the basic mechanism of leukemic cell kill can be elucidated. We have learned that the acute leukemia cell has extraordinary antigenic complexity; the effects of both specific and nonspecific immunotherapy in this disease have been discouraging. However, as we evaluate the effects of therapy on general and tumor-specific immunity, it is hoped that our understanding of mechanisms involved in leukemia and immune surveillance will increase. The central nervous system is a major site for relapse in acute leukemia. Our studies on route and scheduling of CNS chemotherapy promise an effective and relatively nontoxic approach to this important problem. It is further evident that the most important contribution that can now be made to the treatment of "good risk" patients with acute lymphocytic leukemia is to provide effective CNS prophylaxis without the very dramatic toxicity we have detected in association with cranial irradiation. By exploiting the elegant monkey model for CNS pharmacokinetics developed in this Branch, the very sophisticated monitoring techniques available to us (e.g., C.T. scans), and our experience in clinical pharmacokinetics, we hope to develop an effective CNS prophylaxis to be administered systemically and hopefully without significant sequellae.

We are beginning to make progress in the treatment of advanced solid tumors that have been refractory to conventional doses of combination chemotherapy. The response in American Burkitt's lymphoma has been particularly gratifying in that a small number of patients with this disease have been treated with high-dose chemotherapy and have had striking long-term remissions at a point in time when the disease has historically been invariably fatal. The role of autologous marrow rescue in this setting, while tentative, also appears to hold much promise. We further believe that the concept of high-dose combination chemotherapy together with maximal supportive care will be useful in metastatic osteogenic sarcoma, in rhabdomyosarcoma, and in Ewing's sarcoma, although our evidence to date is disappointing in the case of neuroblastoma. In all of these situations, our objective is to debulk the tumor to the maximum extent and then use intensive chemotherapy--prompted by the mathematical reasoning of Norton and Simon that the low growth rate of a small tumor volume mandates a large drug dose. In each of the solid tumors we are exploring other interesting observations: the synergistic possibilities of treatment with cis-platinum and adriamycin in the case of metastatic osteogenic sarcoma, pharmacological debulking in the case of localized rhabdomyosarcoma, and the suggestive efficacy of total body irradiation and prophylactic pulmonary irradiation in the case of Ewing's sarcoma.

We have made considerable progress in the development of multi-resource approaches to the psycho-social concomitants of cancer in patients, their families, and therapists. We believe that this approach is exceedingly important, for failure to deal with these concomitants may be reflected in inadequate treatment of cancer. We believe that the Pediatric Oncology Branch has provided a model for coordinated research and care in the psycho-social concomitants of chronic illness which has proven applicable to many other diseases and institutions.

Finally, it should be noted that certain of our studies relate to clinical concerns that are not restricted to cancer treatment: For example, our Phase I-II trial of interferon inducers has for the first time demonstrated significant interferon induction with minimal toxicity in man. These data should prove useful in a wide variety of infectious disease problems, e.g., control of the hepatitis B carrier state.

Proposed Course:

All of the studies enumerated previously will be continued. Aggressive protocols have been developed for acute leukemia patients who relapse from primary induction protocols but remain sensitive to extant chemotherapy. Increased emphasis will be given to phase I trials, in particular studies of maytansene, poly I-poly C/poly-l-lysine, AMSA, coformycin, and glutaminase. We shall continue to explore the pharmacokinetics of methotrexate so as to improve the therapeutic index of this drug as it is used in the treatment and prophylaxis of meningeal leukemia. Experiments on the use of high-dose chemotherapy in refractory solid tumors will be expanded; in these studies, chemotherapy is assessed in a situation whereby maximum supportive care is possible, including autologous bone marrow rescue. Our interest in laminar-flow-room prophylaxis, granulocyte transfusions, total parenteral nutrition, and empiric antibiosis will continue pari passu with studies of high-dose chemotherapy. We plan to expand studies in clinical and molecular pharmacology and to utilize the results of such studies in planning further clinical chemotherapy trials. Both our clinical and basic studies in the kinetics of hematopoietic reconstitution and differentiation will be used to guide further experiments on clinical marrow reconstitution, using cryopreserved marrow (or stem cells obtainable from the peripheral blood).

In collaboration with the Laboratory of Developmental Psychology, NIMH, we plan to expand our study of stages in normal and abnormal childhood development as they relate to the setting of chronic illness.

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01-CM-06820-04 PO

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7/1/76 - 9/30/77

TITLE OF PROJECT (80 characters or less)

Hematological Support of Patients with Cancer or Aplastic Anemia

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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LAB/BRANCH
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SECTION
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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
12	7	5

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☒ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Hematological support techniques which may be of importance in the treatment of patients with cancer or aplastic anemia are being developed and improved. Studies in patients and in pre-clinical canine models facilitate the development of new methods for the cryopreservation of human marrow, permit a determination of the dose of stem cells which is necessary for bone marrow reconstitution following ablative chemotherapy, and have led to the collection of adequate numbers of stem cells from peripheral blood for autologous reconstitution using automated cell separators. In the area of granulocyte transfusion, we have established the dose of granulocytes necessary to protect an animal from life-threatening pseudomonas sepsis, and are studying ways of achieving this dose in man.

Clinical and in vitro studies are being utilized to clarify which histocompatibility antigens are of the greatest importance, and in the identification of compatible donor-recipient pairs for the purpose of platelet transfusion.

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in vitro assays of platelet compatibility, and histocompatibility phenotyping are conducted. A donor file of 20,000 individuals (all of whom are typed with respect to the A and B loci of the HLA system) is backed up by an interlocking network of 13 centers for donor procurement. Finally, a serum repository with at least 14,000 entries is maintained for clinical and laboratory investigations.

Major Findings:

A. Bone Marrow Transplantation

1. The intravenous infusion of cryopreserved autologous bone marrow can avert the otherwise lethal myelosuppression of total body irradiation in rodents, dogs, and non-human primates. We have developed a canine model in an effort to study factors which may influence the engraftment of cryopreserved autologous bone marrow. Dogs were subjected to 1000 rads of total body irradiation at 9 rads per minute and then supported with fluids and antibiotics alone or with an infusion of their own cryopreserved bone marrow which had been removed and stored in 10% dimethylsulfoxide in the vapor phase of liquid nitrogen prior to irradiation. All six irradiated animals treated without bone marrow infusion died within 9-12 days. All ten animals receiving greater than 0.5×10^8 bone marrow cells/kilogram stored for up to 5 months survived. To study the effect of cell dose on engraftment, thirty-one dogs were given marrow stored for up to five months in cell doses ranging from 0.1×10^8/kilogram to 1.0×10^8/kilogram. Animals receiving greater than 0.5×10^8 bone marrow cells/kilogram engrafted more often and more quickly than those receiving less than this dose ($p<.05$). To determine if prolonged storage affected the engrafting ability of cryopreserved marrow, animals were given 0.5×10^8 marrow cells/kilogram stored for 2, 5, 9, 12 and 18 months. Animals whose marrow was stored for longer times engrafted less often ($p<.001$) and those that engrafted did so more slowly than those whose marrow was stored for shorter periods.

2. A comparison of the rate of hematopoietic recovery in man was conducted following intensive chemotherapy for non-Hodgkin's lymphoma (mainly Burkitt's lymphoma); patients were supported by conventional means or autologous marrow infusions were employed. Infusions of cryopreserved autologous marrow cells (dose was always greater than 0.5×10^8 nucleated cells per kg) resulted in a time to recovery of 1000 wbc/mm^3 of 10-18 days (median 13), while patients not receiving autologous marrow infusions following intensive chemotherapy required 12-38 days (median 23 days) (see report 06810-13 for further detail).

3. Methods for the fractionation of human marrow prior to cryopreservation were developed using the Haemonetics Model 10 Cell Separator. Seventy-eight percent of the stem cells, as measured by the colony-forming unit assay, could be recovered during this fractionation, which reduced the volume to 14%, the hemoglobin content to 10%, the mature myeloid cells to 36%, and resulted in a total cell yield of 40%.

Objectives:

1. To use autologous marrow infusions in support of the intensive treatment of advanced malignancy; to improve methods of cryopreservation of human marrow; to establish the dose of stem cells (measured indirectly by the colony-forming unit assay) necessary for marrow reconstitution following ablative chemotherapy; to collect quantities of stem cells from peripheral blood which are adequate for autologous reconstitution following ablative chemotherapy; and to study the biology of animal and human allogeneic marrow transplantation.

2. To study the dose of granulocytes needed to protect neutropenic patients from life-threatening gram-negative septic episodes (using a variety of granulocyte collection techniques with and without various forms of donor and receipient pharmacological pre-treatment) in the prophylactic and therapeutic setting; to design rotors for the continuous-flow centrifuge such that the numbers of granulocytes which are collected can be increased; to test the effect of increasing the dose of granulocytes in the treatment of gram-negative sepsis in patients who are profoundly neutropenic; to evaluate various in vitro tests of sensitization with respect to their utility in predicting donor-recipient incompatibility in the setting of granulocyte transfusion.

3. To analyse platelet transfusions in which the HLA phenotypes of donors and recipients (and post-transfusion increments) are known, in order to assess the relative importance of each of the HLA antigens in identifying donors for long-term support of patients with cancer or aplastic anemia; to conduct in vitro studies using sera of recipients collected at various times during treatment, and cells of donors, so as to establish mechanisms of incompatibility and assess the HLA system with respect to its efficiency in identifying compatible donors for platelet transfusion.

Methods Employed:

In vitro assays are used to identify the numbers of stem cells present in marrow samples. These assays involve incubation of nucleated marrow cells in semisolid culture media (methyl cellulose or agar). The number of colonies formed in 10-14 days constitutes an indirect measure of the stem cell content. Automated temperature-controlled instruments are used for the cryopreservation of marrow. Aminco continuous-flow centrifuges, Haemonetics discontinuous-flow centrifuges, and the Fenwal filtration leukapheresis device are used for the collection of granulocytes and platelets. This latter component is also collected by conventional non-automated centrifugational methods ("split bag method"). An intramural laboratory equipped with advanced culture and analytical techniques (cytofluorograph, scintillation spectrometers, ultracentrifuges, laminar flow hoods, phase bright-field and fluorescent microscopes) is supported by contract facilities in which canine experiments,

4. We have studied the toxicity and immune suppression of supralethal total body irradiation (800 to 2000 rads, ^{60}Co) at 3 dose intensities (10 rads/min, 49 rads/min, and 100 rads/min). In 79 intensively supported radiation control animals, the $LD_{50(5)}$ (that theoretical dose at which 50% of the animals will die within 5 days) for these dose intensities is estimated respectively to be 1556, 941, and 921 rads. A biomodal pattern of early (median 4 days) and late (median 9 days) deaths was observed corresponding to histopathologic evidence of the intestinal and hematopoietic radiation syndromes. Random donor bone marrow transplants were performed in 83 animals to test immune suppression afforded by 800 rads and 1000 rads at dose intensities of either 10 rads/min or 49 rads/min. Bone marrow cell dose was varied to analyze its effect on engraftment. A greater degree of immunosuppression with less toxicity was achieved at the lower dose intensity. A minimum dose of 3 to 5 x 10^8 nucleated allogeneic bone marrow cells per kg (readily obtainable from living donors) resulted in a high percentage of engraftment with lethal graft vs. host disease following conditioning with 1000 rads midplane at 10 rads/min, the optimum regimen employed.

5. Immunosuppression with methotrexate (MTX) alone or in combination with citrovorum factor (CVR) was evaluated for its toxicity, enhancement of engraftment, and effectiveness in reducing graft-versus-host-disease (GVHD) in lethally irradiated dogs transplanted with bone marrow from unrelated histo-incompatible donors. Three groups of recipients were studied: animals given no immunosuppressive therapy (A); animals given MTX 0.25 mg/kg on day 1, 3, 6, 11 and then weekly until day 102 (AM); and animals given MTX 0.25 mg/kg on day 1, 3, 6, followed by weekly MTX 25 mg/kg through day 102 in combination with citrovorum factor injections beginning the second day the total leukocyte count reached 1 x 10^9 cells/mm^3 (AMC). Engraftment rate, hematopoietic recovery and incidence of GVHD was similar in all three groups. Animals in the AMC group lived significantly longer than the A group ($p<.05$), but no significant difference in survival was seen between the AMC group and the AM group, although toxicity was greater in the AM group with a higher incidence of early deaths ($p<.05$). It is concluded that MTX increases survival and the addition of CVF reduces toxicity without loss of immunosuppression. However, no effect on survival over 30 days was seen with MTX or with the high dose MTX permitted by CVF rescue.

6. The increased utilization of bone marrow transplantation in a variety of clinical situations has focused attention upon the problem of ABO incompatibility among HLA matched, MLC negative donor-recipient pairs. Three techniques (plasma exchange, intravenous administration of soluble specific blood group substances, and transfusion of donor type erythrocytes) were employed in an attempt to transplant six leukemia patients from ABO incompatible siblings. Four of five evaluable patients engrafted after successful reduction of preformed

isoantibodies. Our results document that it is possible to transplant across the ABO barrier. In light of these results, ABO incompatible bone marrow transplantation should be considered when no other suitable donor is available.

B. Granulocyte Transfusion

1. A canine model was devised to evaluate the quantitative aspects of granulocyte transfusion. Beagle dogs were given cytoxan (40 mg/kg) as a single bolus, which rendered them neutropenic within 5 days. All animals were supported with ampicillin, gentamicin, clysis, and platelet infusions. Pseudomonas (1×10^8/kg) was infused on the fifth day following cytoxan and dogs were then assigned to a control group or to receive daily granulocyte transfusions, in addition to fluids and antibiotics. The dose of granulocytes, as collected by the continuous-flow centrifuge, needed to protect neutropenic dogs challenged with a lethal dose of pseudomonas, was found to be 2×10^8/kg. Two and one-half times as many granulocytes were needed to protect dogs, in an identical model, when the granulocytes were collected by nylon filter-adhesion leukopheresis. The increased dose of granulocytes (as collected by adhesion leukopheresis) needed for protection was thought to be due to changes in chemotaxis and bactericidal function known to attend the adhesion of granulocytes to nylon filters. This canine model will be used to study the effects of donor and recipient pre-treatment on the dose-dependence of granulocytes.

2. A series of granulocyte collections is underway to evaluate a new rotor design for use in the Aminco cell centrifuge. Preliminary results suggest that a 2-4 fold increase over yields of granulocytes obtainable with conventional rotors may be achievable.

3. A series of granulocyte collections, using the Fenwal leukopak (adhesion leukopheresis), was carried out in a series of 5 healthy male volunteer donors. Two collections were made from each donor: a control collection, and a collection preceded by treatment with a total of 2.0 mg of colchicine. This study was designed to evaluate what protective effect colchicine pre-treatment might have on the function of granulocytes collected by the filtration leukopheresis method. Granulocytes collected from donors without colchicine pre-treatment exhibited a 50% reduction in bactericidal and chemotactic function when compared to donor granulocytes before collection. Colchicine administration totally prevented this qualitative defect in granulocyte function. Colchicine pre-treatment was associated with a 30% reduction in absolute yield of total leukocytes. Further studies are now underway to ascertain if granulocytes from colchicine pre-treated donors exhibit normal survival and chemotaxis *in vivo*.

4. An analysis of the utility of *in vitro* assays in predicting post-transfusion increments and transfusion reactions in the clinical use of granulocytes was conducted by reviewing the course of 150 granulocyte transfusions in 10 neutropenic septic patients. Each transfusion

was studied by assays of granulocytotoxicity, lymphocytotoxicity, microleukoagglutination and capillary leukoagglutination. Recipient reactions and post-transfusion increments were recorded. No statistically significant relationship existed between post-transfusion increments and the positivity or negativity of any of these assays. The capillary leukoagglutinin assay was positive in 57% of the transfusions. The positivity of this assay bore no relationship to the occurrence or the severity of reactions, even when the titre of the assay was studied. As no life-threatening transfusion reactions occurred in the series of patients studied, we cannot rule out at this time that the presence of extremely high titres of leukoagglutinins by this test would not be predictive of incompatibility. The remaining three assays used were negative in over 95% of the transfusions. At the current time, we are recommending prophylactic pretreatment with steroids if the capillary leukoagglutinin reaction is greater than 50% positive at a dilution of 1/32. These studies indicate that the currently available in vitro tests for determination of donor-recipient compatibility for granulocyte transfusion are inadequate.

5. Twenty-seven patients with granulocytopenia ($<1000/\mu l$) were prospectively randomized for 30 episodes of documented gram-negative septicemia. Survival of the control group, receiving an identical conventional appropriate antibiotic regimen alone, was 5 of 14 compared to 13 of 16 in the granulocyte transfusion group ($p<0.02$). The two populations were comparable for factors known to influence survival (age, disease, duration of granulocytopenia, causative organism). No difference in survival was noted for patients transfused with granulocytes procured by continuous-flow centrifugation or filtration leukopheresis, although transfusion reactions with the latter were more common. Survival was not related to HLA match nor to post-transfusion increment. An important factor in determining the outcome of treatment for sepsis was recovery of bone marrow function (return of granulocyte count $>1000/\mu l$). Eighty-three percent (5 of 6) of the control group and all (4 of 4) of the transfusion group, with recovery of peripheral granulocytes, survived the episode of sepsis. In contrast, none (0 of 8) of the control patients compared to 75% (9 of 12) of the transfusion group survived despite persistent granulocytopenia ($p<0.001$). Daily compatible granulocyte transfusions appear to be additive to appropriate antibiotic regimens in the treatment of gram-negative septicemia which occurs as the result of granulocytopenia.

6. Although granulocyte transfusion therapy has been shown to be effective in infected granulocytopenic animals and humans, the relative effectiveness of granulocytes (PMN) harvested by continuous flow centrifugation (CFC) or by continuous flow filtration leukapheresis (FL) remains uncertain. Studies in vitro of morphology and granulocyte function have suggested cells collected by FL may be damaged. To compare the

function *in vivo* of granulocytes collected by different methods, dogs were made granulocytopenic with cyclophosphamide (CYT) and then transfused with granulocytes collected by CFC or FL. The local neutrophil mobilization (LNM) through a standard skin abrasion into a chamber containing a strong chemoattractant, autologous serum, was measured. Greater LNM was found after transfusions of CFC PMN than after transfusions of the same number of FL PMN ($p<0.003$). This difference persisted even when the dose of FL PMNs was four times greater than that of CFC PMN and when the FL donor was pretreated with steroids ($p<0.001$). These results suggest that during filtration leukapheresis, granulocytes are functionally altered and that their function *in vivo* may be compromised.

C. Platelet Transfusion

Over 1000 platelet transfusions have been analyzed in which post-transfusion increments and the HLA phenotypes of the donor and recipient were known. Particular emphasis was placed on transfusions to patients with aplastic anemia who were not infected or undergoing other procedures at the time of the transfusions. The effectiveness of transfusions from a single donor when given to the same recipient was followed over time to determine whether decreasing post-transfusion increments would occur in donor-recipient pairs in which there was at least a single HLA antigen mismatch. In our series, a pattern of decreasing post-transfusion increments consistent with sensitization was seen when a single donor-recipient pair was mismatched at the HLA locus W21. Possible sensitization patterns were seen with single antigen mismatches involving the loci W33, 29 and 13, but the number of transfusions was too small for these studies to be conclusive. No definite patterns of sensitization were seen when donor-recipient pairs were mismatched at W23, W24, W32, W22, or W12/35. When platelets from the donors were tested for the presence of these specificities, the appropriate antigens (or antigens cross-reacting with antisera presumed to be specific for these antigens) were found. At least two possible conclusions can be drawn from these studies: Efficient use of the HLA phenotyping system for the identification of compatible donor-recipient pairs must be based on a knowledge of the cross-reacting specificities of the antisera used for the phenotyping. Alternatively, there may be other phenotypes on platelets, as yet not identified, which are of importance in identifying compatible donor-recipient pairs for platelet transfusion. Studies are currently underway to determine which interpretation of the data is correct.

Significance to Biomedical Research and the Program of the Institute:

Hematological support is the cornerstone upon which long-range, intensive therapy of patients with malignancy or aplastic anemia is based. The advances we have made in the use of autologous marrow infusion may permit the use of new and more intensive regimens of drugs for the treatment of resistant tumors. These studies suggest that human marrow may be cryopreserved and infused safely after prolonged storage, and that its use may be associated with accelerated hematopoietic recovery. Our current studies with granulocyte transfusion have provided a pre-clinical model in which many of the important issues pertaining to the use of this blood component may be studied. Colchicine pre-treatment appears to improve the function of grnaulocytes collected by adhesion leukopheresis, and this observation may suggest further methods of protection of granulocytes from the effects of nylon filters. Finally, our increased technical ability to collect large numbers of granulocytes may allow us to test, in a clinical trial, the possible advantage of larger numbers of granulocytes in the treatment of gram-negative sepsis in neutropenic patients. All of these granulocyte studies have taken on particular significance with our recent demonstration that white cell transfusions indeed benefit septic, neutropenic patients -- but only those with severely protracted marrow recovery kinetics. Our studies on the role of HLA antigens in platelet transfusions may be very important, for this blood particle is by far the most frequently needed component for the support of cancer patients and the cost of providing HLA matched platelets to every patient is enormous. Hopefully, our studies will help us identify ways in which to make this important system function more efficiently at a lower cost.

Proposed course:

Emphasis will be placed on development of the capability for collection of adequate numbers of stem cells from the peripheral blood in order to achieve hematopoietic reconstitution following therapy with doses of agents which are ablative (or nearly ablative) to hematopoietic cells. Such a capability would permit delivery of intensive chemotherapy through multiple successive courses. These experiments will utilize initially a pre-clinical canine model and subsequently be applied to normal human donors and ultimately to patients who are candidates for intensive chemotherapy.

An attempt will be made to assess the effect of the dose of granulocytes, infused into profoundly neutropenic patients in whom gram-negative sepsis has been documented, on the rate of resolution of this sepsis. New improved granulocyte collection techniques based on adhesion leukopheresis will be a primary area of interest because of the potential importance of these techniques in the therapy of septic neutropenic patients. Finally, a randomized trial of HLA matched vs. non-matched platelet tranfusions will be carried out to clarify in which groups of patients the HLA matching system is necessary to identify compatible donor-recipient pairs.

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15. Kauffmann, J.C., Alter, H.J., Graw, R.G., Jr., McGinniss, M.H., Herzig, G.P., Bull, M.I., Decter, J.A., Herzig, R. H., Lohrmann, H.P., Gorin, N.C., and Gratwohl, A.A.: ABO incompatible bone marrow transplantation. Blood, in press.

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17. Oldham, R.K., Oran, J.A., Cannon, G.B., Ortaldo, J.R., Dunston, G., Appelbaum, F.R., McCoy, J.L., Djeu, J., and Herbermann, R. B.: Cryopreservation of human lymphocyte function as measured by in vitro assays. Int. J. Cancer 8:145-155, 1976.

CM-06820-04 PO

Appendix A

Contract Number: NOL-CM-43702

Contractor: Hazelton Laboratories of America, Inc.

Principal Investigator: Charles Bowles

Budget: $408,580

Man Years: 10 (4 = professional, 6 = other)

Objective:

Preclinical investigation of leukocyte transfusions and autologous marrow infusions.

Finding:

The preclinical canine model was used to establish that a daily dose of 2 x 10^8/kg of granulocytes collected by the continuous flow centrifuge is necessary to treat gram-negative sepsis in severely neutropenic dogs receiving antibiotics and fluids following injection of 1 x 10^8/kg pseudomonas organisms intravenously. A daily dose of 5 x 10^8/kg of granulocytes is required when they have been collected by adhesion leukopheresis. Studies conducted in the canine bone marrow autograft model (Total body irradiation followed by cryopreserved autologous marrow infusion) have established that a dose of 0.5 x 10^8/kg nucleated marrow cells is needed for hematopoietic reconstitution.

Plan:

The same canine autologous reconstitution model will be used to establish the number of stem cells (indirectly measured by the CFUC assay) necessary for hematopoietic reconstitution. The canine model will also be used to develop the technical capability to collect adequate numbers of stem cells from the peripheral blood by continuous flow centrifugation , so as to reconstitute the marrow following total body irradiation.

Publications: None

Appendix B

Contract number: NOL-CM-63810

Contractor: Microbiological Associates

Principal Investigator: Robert Trapani, Ph.D.

Budget: $100,000

Man Years: 3.29 (2.22 = professional)

Objective:

To perform crossmatch studies prior to transfusions of platelets and leukocytes, and to store serum on patients in the Pediatric Oncology Branch.

Findings:

Studies of over 100 granulocyte transfusions have indicated that in vitro assays of lymphocytotoxicity, granulocytotoxicity and leukoagglutinins are of no use in predicting donor recipient incompatibility in granulocyte transfusions. The above in vitro assays have also been used in the study of HLA matched and mismatched platelet transfusions. None of these tests or an anti-platelet antibody technique have shown any relationship to the presence or absence of apparent sensitization in donor-recipient pairs during long-term platelet support.

Plan: Unchanged

Publications: None

Appendix C

Contract Number: N01-CM-02092

Contractor: UCLA

Principal Investigator: Paul I. Teraskai, Ph.D.

Budget: $378,512

Man Years: 12.9 (8.1 = professional)

Objective:

This is a service contract which provides HLA-typing for patients, families, and blood donors for NCI patients (Clinical Center and Baltimore Cancer Research Center). The contract also serves the computer processing required for the national platelet donor pool.

Findings:

See narrative in annual report concerning numbers of donors HLA-typed and the status of the national donor pool.

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01-CM-06830-07 PO

PERIOD COVERED
7/1/76 - 9/30/77

TITLE OF PROJECT (80 characters or less)

Infectious Complications of Malignancy: Prevention, Diagnosis and Therapy

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	P. A. Pizzo, Senior Investigator	PO C
	A. S. Levine, Chief, Pediatric Oncology Branch	PO C
OTHER:	F. A. Gill, Assistant Chief, Microbiology Service	CP CC
	J. D. Maclowry, Chief, Microbiology Service	CP CC
	F. G. Witebsky, Assistant Chief, Microbiology Service	CP CC
	M. Weiner, Assistant Professor	Univ. of Texas
	S. Siegel, Associate Professor	Univ. of S. Calif.
	H. Wright, Chief, Infectious Disease Service	Univ. of S. Calif.
	E. J. Sussman, Visiting Fellow	LDP M
	R. M. Simon, Head, Biostatistical Inform. Systems Unit	CO C

COOPERATING UNITS (if any)
Laboratory of Clinical Investigation (NIAID); Clinical Pathology and Nuclear Medicine Departments (CC): Department of Pediatrics, University of Southern California; Biostatistical Inform. Systems Unit, NCI; Lab. of Developmental Psych.M.

LAB/BRANCH
Pediatric Oncology Branch

SECTION
Infectious Disease Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS: 6	PROFESSIONAL: 4	OTHER: 2

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Infection is the major cause of morbidity and mortality in patients with malignant diseases. We are developing and evaluating the utility of various techniques and procedures to reduce the incidence of severe infection in cancer patients and other compromised hosts. The primary prophylactic technique under study is the laminar-air-flow type of isolation-filtration unit. We wish to learn whether this unit reduces the incidence of infection during chemotherapy, if patients undergoing such protection can tolerate more intensive chemotherapy, and if they consequently have an improved anti-tumor response. Other areas of interest include the improvement of methods by which occult infectious agents may be detected and eradicated; an evaluation of the acquired germ-free state and the influence of this state on physiologic and metabolic processes; and an evaluation of newer anti-microbial agents. In prospective trials, we are assessing the current etiology of febrile episodes in cancer patients, the most appropriate workup for fever in such patients, and the efficacy and toxicity of empiric antibiotic regimens offered for varying periods of time. Other studies concern the radioimmunoassay for candida antigen.

Objectives:

1. To assess the current etiology of febrile episodes in patients with malignancy, and the relationship of these episodes to cancer treatment, prior infection, degree of host compromise, and granulocytopenia.

2. To determine the most appropriate means for evaluation of fever in the compromised host by assessing invasive and noninvasive diagnostic techniques.

3. To improve methods by which occult infections may be detected and eradicated.

4. To assess the efficacy and toxicity of empiric antibiotic regimens, especially when administered for prophylaxis during periods of profound granulocytopenia.

5. To assess the utility of various techniques and procedures designed to reduce the incidence of infectious complications in patients with neoplastic diseases and in other compromised hosts.

6. To evaluate the utility of the "total protected environment" in reducing the incidence of infection in patients with solid tumors undergoing ablative chemotherapy (with or without autologous bone marrow rescue), and to learn whether such protection permits the administration of more intensive chemotherapy and results in improved tumor responsiveness and prolonged survival.

Methods Employed:

Patients developing a fever are rigorously assessed for a possible infectious etiology and their subsequent course is carefully followed. Patients receiving empiric or prophylactic systemic antibiotics are monitored with pharmacokinetic and metabolic assays for potential toxicity from these antibiotics, and microbiological surveillance for alterations in microbial sensitivity is carried out. Newer diagnostic studies include gallium scintillation scanning and a radio-immunoassay for candida antigens.

Assessment of the total protected environment [laminar-air-flow-room (LAFR), oral nonabsorbable antibiotic prophylaxis, cutaneous and orificial antisepsis] includes serial patient and environmental microbial surveillance as well as longitudinal assessment of physical compliance and psychological tolerance.

Major Findings:

A. The relationship of fever, granulocytopenia, and antimicrobial therapy to bacteremia was studied retrospectively in 53 cancer patients. Severe granulocytopenia was present at the time blood cultures were positive in 27 of 31 episodes of bacteremia. Twenty-five episodes of bacteremia were documented before the initiation of antimicrobial therapy in patients who were granulocytopenic and febrile. No bacteremia occurred in the absence of fever. Only two bacteremias occurred while patients were receiving parenteral antimicrobials. Antimicrobial therapy was terminated 30 times in the presence of granulocytopenia and fever, and subsequent bacteremia occurred in 14 patients within 4 days. Patients who died with fungal disease did not receive more antibiotics than patients who died without fungal disease. These data suggest a rationale for long-term use of antimicrobial therapy in patients with persistent granulocytopenia and fever.

B. Fever and Antimicrobial Therapy Study - As a consequence of the retrospective study described above, all POB patients developing a fever are now entered on a new prospective study. Patients who are also granulocytopenic (PMN <500/mm^3) commence empiric broad spectrum antibiotics (KGC: keflin, gentamicin, carbenicillin) following initial evaluation and culturing. If no infectious etiology is discerned, these antibiotics are continued for seven days, and at that time the patient's granulocyte count and fever course determine whether the patient is randomized to discontinue antibiotics or to continue further antibiotic therapy while the PMN remains <500/mm^3. Patients who are both granulocytopenic and febrile after seven full days of antibiotic administration may also be randomized to receive empiric amphotericin in addition to broad spectrum antibiotics. Patients whose initial evaluation reveals an infectious source, or who subsequently develop a proven infection, are treated with appropriately specific agents. To date 229 febrile episodes (occurring in 118 patients) have been recorded in this protocol. Twenty-eight of these episodes occurred in patients who were not granulocytopenic. The remaining 201 episodes (88%) occurred in patients who were granulocytopenic (PMN <500/mm^3), suggesting that granulocytopenia is strongly associated with subsequent fever in this patient population. Initial evaluation revealed a clinically or microbiologically demonstrable source of infection in 105/201 episodes (52%). Evaluation of the remaining 96 episodes failed to reveal an infectious etiology initially, and all such patients were begun on empiric broad spectrum antibiotics (KGC) following the initial evaluation. In 47 episodes (49%), there was restoration of marrow function during the first seven days of antibiotic treatment, and all of these patients recovered without sequelae when antibiotics were discontinued. Granulocytopenia persisted during the first seven days of KGC in the remaining 49 cases (51%). Twenty-eight of the patients with persistant granulocytopenia became afebrile during these first seven days of KGC. This group of patients was randomized to either discontinue antibiotics (11 episodes) or to continue KGC (14 episodes). In four of the 11 cases (36%) in which antibiotics were discontinued (at a time when the patient was afebrile and granulocytopenic), fever recurred within 12 hours to five days (two with a documented infectious source); all four patients responded to the reinstitution of antibiotics. In contrast, all of the patients in whom antibiotics were randomly continued during the period of granulocytopenia recovered without any infectious sequelae.

Twenty-four patients remained both febrile and granulocytopenic after seven days of empiric antibiotics. Nine of these were randomized to discontinue antibiotics, nine to continue KGC alone, and six to continue KGC along with empiric amphotericin. Of the patients randomized to discontinue antibiotics, four developed a clinically and/or microbiologically demonstrable infection, three died from noninfectious causes, and two recovered from the episode without sequelae. Of the nine patients randomized to continue KGC alone, one developed a disseminated fungal infection, and a second patient developed sepsis with an organism resistant to KGC. A third patient died from P. carinii pneumonia (which had been diagnosed prior to beginning antibiotics). Five other patients randomized to continue KGC recovered without sequelae, and one died from noninfectious causes. All of the six patients who were randomized to KGC plus amphotericin recovered from the episode. These observations, while preliminary, suggest that a major determinant for fever and infection in the patient with cancer is the presence of granulocytopenia. The data also suggests that infectious complications comprise the majority of febrile episodes in the granulocytopenic patient. It appears clear from this data that patients whose granulocyte count recovers within the first seven days after the onset of the febrile episode may recover without sequelae after a short course of antibiotic therapy. It is also suggested that continued broad spectrum antibiotic administration during periods of granulocytopenia (including amphotericin) may be useful.

Other observations of interest are that the last two years has witnessed a significant change in the pattern of septicemia such that gram-positive organisms (especially Staph and Strep) now comprise the majority of infections in our patient population. This is unaccompanied by any evidence for horizontal transmission. It also appears that the absolute incidence of gram-negative infections in our patient population has decreased, and if this pattern continues, it would have a significant impact upon the design and interpretation of supportive care studies. Surveillance studies are also in progress to assess the significance of colonization by gram-positive organisms (Staphylococcus) during subsequent periods of granulocytopenia. Finally, it appears that the incidence of septicemia and serious infections in patients with leukemia or solid tumors is now comparable. This most likely relates to use of more intensive chemotherapy for solid tumor patients, with its consequent prolongation of granulocytopenia.

C. Protected Environment Study:

Forty patients ranging in age from three to thirty years have been treated with high-dose chemotherapy for metastatic neuroblastoma, osteosarcoma, rhabdomyosarcoma, Ewing's sarcoma, non-Hodgkin's lymphoma, and leukemia. All of these patients were apparently resistant to conventional-dosage therapy. Patients were treated either in a LAFR (26 patients) or a conventional ward setting (14 patients). Patients receiving autologous marrow rescue were randomized to either the LAFR or control group. The incidence of fatal infections has been low in both the LAFR and control groups (one in each group). Infections which occurred in LAFR patients reflect the current limitations of the germ-free state in man, these infections being predominately due to organisms persistent in the mouth--an area notoriously difficult to sterilize. Our experience shows that the techniques of protected isolation and intensive chemotherapy can be used in young children and adolescents. Both the tolerance for isolation and its impact on the patient and family are being prospectively assessed, and to date no child has endured psychological complications which have necessitated removal from an isolation unit. Both control and LAFR patients received intensive combination chemotherapy. The data is too preliminary to permit a comparison of the results between the two groups of patients. Considering the entire group of 40 patients, five have attained complete remission which was sustained for greater than six months, four had a short complete remission, and nine patients had partial remissions of short duration. Fourteen other patients showed no significant response to chemotherapy and eight others are not evaluable at this time. Hence it appears that 18 patients (56%) responded to intensive chemotherapy. However, this response was lasting in only five patients. On the other hand, toxicity associated with these regimens (excluding the infectious complications) has been significant. Hence, while the data suggest that tumors which appear to be drug-resistant may respond to higher levels of chemotherapy, it is probable that this will lead to prolonged survival in only a small number of patients. Whether this trend can be improved by modifications of drug scheduling is currently being explored.

Significance to Biomedical Research and the Program of the Institute:

One of the major sequelae of chemotherapy is bone marrow depression resulting in significant granulocytopenia. Such granulocytopenia significantly increases the incidence of life-threatening infectious complications in the cancer patient. Consequently, supportive care of the patient is essential if the potential benefits of chemotherapy are to be achieved. This implies not only an understanding of the natural history of infectious complications, but also the development of treatment schedules which provide maximum benefit and safety for the patient. Since our studies suggest that escalation of chemotherapy may improve the response rate for patients resistant to conventional doses of chemotherapy, and since the risk of infections is a major impediment to such intensification, the development of programs which reduce the incidence of infection during periods of granulocytopenia is essential. Our studies to date suggest that regimens using prophylactic antimicrobial therapy may be helpful in lessening the infectious morbidity and mortality related to protracted granulocytopenia. This affords the opportunity to explore new chemotherapeutic strategies which may provide a higher therapeutic index.

Proposed Course:

We shall continue our major studies on fever and antibiosis in the cancer patient, and our assessment of the utility of protected environments in the treatment of various pediatric solid tumors. In our fever and antimicrobial therapy study, it is anticipated that a larger number of patients will be accrued over the coming months, since patients from the Los Angeles Children's Hospital will also be treated according to our protocol.

The parenteral combination of trimethoprim-sulfamethoxazole (Bactrim) will also be investigated. This agent has proven useful for the treatment of some organisms resistant to aminoglycosides and penicillins, and has more recently been shown to have utility in the treatment and prophylaxis of Pneumocystis carinii pneumonia. While the oral preparation has had more common usage, it has been noted that absorption tends to be erratic, especially in severely ill patients. Consequently we are exploring the intravenous preparation of this antibiotic for the treatment of infections in cancer patients, as well as assessing its pharmacokinetics in various clinical settings.

Publications:

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01-CM-06840-02-PO

PERIOD COVERED
7/1/76-9/30/77

TITLE OF PROJECT (80 characters or less)

Tumor Immunology and Immune Surveillance

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	D. G. Poplack, Senior Investigator	PO C
OTHER:	S. Ladisch, Clinical Associate	PO C
	R. M. Blaese, Head, Cellular Immunology Section	MET C
	S. Broder, Senior Investigator	MET C
	A. Muchmore, Senior Investigator	MET C
	D. Nelson, Senior Investigator	MET C
	E. C. Lawrence, Clinical Associate	MET C
	D. Mann, Senior Investigator	I C
	J. Smythe, Department of Medicine	Royal Marsden Hospital

COOPERATING UNITS (if any)

Metabolism Branch (NCI), Immunology Branch, NCI; Dept. of Medicine, Royal Marsden Hospital, England

LAB/BRANCH
Pediatric Oncology Branch

SECTION

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
5	3	2

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

In this project, mechanisms of normal cellular immunity and tumor immunology are studied: immunological surveillance of cancer patients and the effects of chemotherapy and immunotherapy on the immune response are explored. We are characterizing acute lymphocytic leukemia according to the immunological subtypes of lymphoblasts and the capacity of lymphoblasts to express immunoregulatory helper and/or suppressor functions in vitro. The role of B cell alloantigens in lymphoblastic leukemia is also under investigation. Particular emphasis is placed on immunologic study of the monocyte-macrophage system (including development of new clinical assays of human monocyte function) in malignancies and immunodeficiency diseases, in the newborn, and in malignant histiocytosis. We are also investigating serum suppressors of cellular immunity in cancer patients, and the role of immune adjuvants and their ability to stimulate granulocyte recovery following marrow-suppressive chemotherapy.

Project Description:

Objectives:

1) To assess the effect of chemotherapy and immunotherapy on the host immune response.

2) To develop methods of distinguishing between immunological subtypes of leukemic lymphoblasts.

3) To determine the effect of chemotherapy on the recovery of granulocytes following chemotherapy-induced granulocytopenia.

4) To assess whether acute leukemic lymphoblasts are capable of manifesting immunologic "helper" and/or "suppressor" function in *in vitro* immunologic assays.

5) To determine the relationship of B cell alloantigens to leukemic lymphoblasts.

6) To develop a clinically relevant test of human monocyte function.

7) To evaluate the role of the monocyte-macrophage in the histiocytic syndromes.

Methods and Major Findings:

A. Immune surveillance of leukemia patients.

Serial immune evaluation has been performed on patients who, while on protocol 72-1 (initial treatment of acute lymphoblastic leukemia), received maintenance chemotherapy according to one of three treatment arms designed to compare the effectiveness of chemotherapy versus chemotherapy plus immunotherapy. Those patients who received BCG immunotherapy developed a vigorous skin test response to PPD; however all other recall skin test responses remained stable throughout the study. *In vitro* lymphocyte responses to specific antigens and non-specific mitogens progressively declined with time. These findings suggest that the recognition and memory functions of the immune system remain intact during chemotherapy, but the proliferative response is impaired. We have extended this serial immune evaluation in patients who successfully completed chemotherapy on the 72-1 protocol. Examination of afferent and efferent cellular immune function has revealed that within one month following the cessation of chemotherapy, immune responsiveness of patients returns to normal. Six-twelve months later, immune responsiveness is also intact. Thus, the immunosuppression which occurs during chemotherapy is rapidly reversed following cessation of treatment.

B. Adenosine Deaminase in lymphoblasts.

We have shown that adenosine deaminase (ADA) levels in blast cells of patients with acute lymphocytic leukemia are elevated when compared to levels in normal lymphocytes. This observation prompted us to study the possibility of a correlation between ADA activity and lymphoblasts differentiated on the basis of their cell surface markers. Lymphoblasts from untreated patients with ALL were evaluated for the presence of sheep erythrocyte and compliment receptors using standard E and EAC rosetting techniques. In addition, sonicated extracts of these Ficoll hypaque-separated lymphoblasts were assayed spectrophotometrically for ADA activity. Of 23 patients tested, 9 had T lymphoblasts (E rosette positive) and 14 had null lymphoblasts (E and EAC rosette negative). The mean ADA activity was 3 times higher in T lymphoblasts than in null lymphoblasts. Thus, there is a significant ($<p.02$) positive correlation between elevated ADA levels and T cell-ALL. This method of distinguishing between immunological subtypes of lymphoblasts is potentially of prognostic and therapeutic value.

C. Stimulatory effect of BCG on granulocyte recovery.
We have conducted studies designed to determine whether immunotherapy stimulates the recovery of granulocytes following leukopenia-inducing chemotherapy. Using an animal (mouse) model, we found that pretreatment with the immunoadjuvant BCG resulted in a more rapid recovery of the leukocyte count following treatment with cyclophosphamide. We have also found that the more rapid recovery in BCG pretreated mice is on the basis of stimulation of the granulocyte series alone. We are currently investigating whether the stimulatory effects of BCG upon the recovery of granulocytes is the result of a direct effect on stem cells, or an indirect effect mediated through enhancing substances such as colony-stimulating factor.

D. Suppressor activity in T-cell leukemia.
Since it has been shown that T lymphocytes are capable of manifesting either "helper" or "suppressor" modulatory effects on *in vitro* immunoglobulin biosynthesis, we are studying whether lymphoblasts from patients with T cell-ALL are capable of this type of immunoregulatory activity. Preliminary studies of one patient with T cell leukemia have indeed demonstrated the presence of suppressor activity. The possibility that this *in vitro* finding has a clinical correlate is suggested by recent observations of immunoglobulin abnormalities in patients with acute lymphoblastic leukemia.

E. B cell alloantigens.
A new system of B cell alloantigens has been investigated by Dr. Dean Mann. We are determining the distribution of these B cell alloantigens on lymphocytes from patients with a variety of disorders, including acute lymphoblastic leukemia. We are especially interested in twins with ALL, since they may allow us to accurately determine the genetics of transmission of the B cell alloantigens.

F. Monocyte-mediated antibody-dependent cellular cytotoxicity.
The lack of a simple, rapid, and quantitative test of the functional activity of the monocyte has hampered studies of the contribution of this cell type to host defense and human disease. We have developed an assay

of antibody-dependent cellular cytotoxicity which depends exclusively upon the monocyte as the effector cell and therefore provides a convenient test of monocyte function. In this system, mononuclear leukocytes (MNL) obtained by Ficoll-Hypaque separation of whole blood are cytotoxic for ^{51}Cr-labeled human erythrocyte targets coated with anti-blood group antibody. Removal of phagocytic monocytes from the MNL by iron ingestion, followed by exposure to a magnetic field, completely abolishes all cytotoxic activity from the remaining MNL population. Similarly, in severely monocytopenic patients with aplastic anemia, cytotoxic effector activity is absent. In normals and less severely monocytopenic aplastic anemia patients, cytotoxicity correlates significantly ($p<0.001$) with monocyte number. Application of this monocyte-mediated antibody-dependent cellular cytotoxicity assay to the study of patients with the Wiskott-Aldrich syndrome has revealed defective monocyte cytotoxic activity in spite of normal monocyte numbers, suggesting that this test may be useful for the assessment of monocyte function in a variety of clinical situations.
In addition, we have studied monocyte-mediated antibody-dependent cellular cytotoxicity in newborn children in an effort to determine whether their monocyte function is normal. Thus far, it appears that this is the case; since other aspects of monocyte function in newborns have been abnormal, it may be that effector function is relatively unique in this regard.
We have also studied the mechanisms of monocyte-mediated cytotoxicity. The defects noted above do not seem to be the result of abnormal FC receptors.

G. Studies on familial lymphohistiocytosis.
Since the monocyte-macrophage is the principle cell involved in the histiocytic syndromes, we have investigated patients with these disorders in an attempt to learn whether the monocyte-macrophage system functions abnormally. We have studied three patients with the syndrome of histiocytosis X, all of whom appear to possess normal monocyte-macrophage function. However, four patients with lymphohistiocytosis (a fatal familial syndrome characterized by abnormal accumulation of non-malignant histiocytes) were found to be anergic to recall skin test antigens. In vitro evidence of abnormal cellular immune function included depressed lymphocyte proliferative responses to specific antigens and impaired monocyte-mediated cytotoxic function. We have found that plasma from patients with lymphohistiocytosis suppresses the responses of normal lymphocytes. On further investigation, it was found that the suppressive plasma was hyperlipidemic; moreover, the degree of in vitro immunosuppression by these plasmas correlated with the degree of lipid elevation. Since an association between hyperlipidemia and suppression of in vitro immune responsiveness has recently been reported, hyperlipidemia may be in part responsible for the immune defects in these patients.

Significance to Biomedical Research and the Program of the Institute:

Our immunological studies have helped to assess the effects of chemotherapy and immunotherapy on the immune system of patients with malignancy. Since, in recent years, it has been shown that the macrophage-monocyte system plays a central role in the host defense against tumor and infection, the development of assays of human monocyte function and the confirmation of their ability to detect clinical abnormalities are of singular importance in permitting further understanding of the monocyte-macrophage system in human disease. Our studies on characterization of leukemic cells using immunological and biochemical markers may be of prognostic and therapeutic importance. Evaluation of the immunologic function of these cells may lead to new approaches in the chemotherapy and immunotherapy of patients with acute lymphoblastic leukemia. Studies on the ability of immunotherapeutic agents potentially to stimulate the recovery of granulocytes following chemotherapy-induced marrow suppression may be of value in the treatment of patients with malignancies.

Proposed course:

Immune surveillance of patients with malignancies will be continued, with particular emphasis on assessment of monocyte-macrophage function in these individuals. Further biochemical and immunological characterization of acute leukemia lymphoblasts will be pursued in an effort to determine the prognostic and potential therapeutic applicability of the adenosine deaminase, T-cell suppressor, and B-cell alloantigen findings discussed previously. Further investigation into the role of the macrophage-monocyte in malignant histiocytic disorders will be pursued. Our findings on the granulocyte-stimulating qualities of certain immunotherapeutic agents will be tested in larger animal models and considered for appropriate human trials.

Publications:

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2. Gerety, R., Poplack, D.G., Hoofnagle, J.H., Barker, L.F., and Blaese, R.M.: Hepatitis B virus infection in the Wiskott-Aldrich syndrome. J. Pediat. 88: 561-564, 1976.

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14. Sher, N.A., Poplack, D.G., Blaese, R.M., and Chaparas, S.D.: The effect of corynebacterium parvum, methanol-extraction residue of BCG, and levamisole on macrophage random migration, chemotaxis, and pinocytosis. J. Natl. Cancer Inst., in press.

Appendix A

Contract Number: N01-CM-12127

Contractor: Hebrew University, Jerusalem, Israel

Principal Investigators: Dr. David Weiss and Dr. Gaby Izak

Buget: $107,450

Man Years: 3

Objectives:

To investigate the therapeutic effect of MER (methanol-extraction-residue of BCG) in patients with acute myelogenous leukemia and to monitor the effect of this agent on the immune response of patients and a variety of animals with tumors.

Methods:

1. A controlled clinical study was undertaken of the therapeutic efficacy of MER as an adjuvant to maintenance therapy in acute myelogenous leukemia.
2. The role of MER in stimulating the immune response was assessed; study of this agent in the therapy of mouse leukemia and guinea pig hepatomas was also undertaken.

Major Findings:

A total of 55 patients has been entered in the clinical study. Forty of those individuals were reported to have achieved remission. According to the assessment of the investigators, there was a significant ($p<0.01$) improvement in mean survival and remission duration in the MER-plus-chemotherapy group as compared to the chemotherapy-alone group. However, our analysis of the submitted patient data failed to confirm these conclusions. Reappraisal of this clinical trial, performed in conjunction with our Clinical Trials Committee, has revealed a number of serious design flaws which make conclusions as to the efficacy of MER premature. We are especially concerned with the facts that the trial, though intended to be randomized, was not; the criteria used by the investigators to define "partial remission" are weak; induction and maintenance chemotherapy was changed at least four times during the study; stratification of patients in terms of important prognostic criteria was inappropriate; statistical methods may not be valid. Although the laboratory aspects of this contract were considered sound, it was decided to terminate the entire contract because of problems regarding the clinical protocol.

Plan:

The contract will be terminated. A six month "phase-out" period will be allowed to provide for an appropriate transition period.

Publications:

1. Ben-Efraim, S., and Diamantstein, T.: Mitogenic and adjuvant activity of a methanol-extraction-residue (MER) of tubercle bacilli on mouse lymphoid cells _in vitro_. _Immunol_. _Communic_. 4: 565-575, 1976.

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14. Weiss, D.W., Kuperman, O., Fathallah, N., and Kedar, E.: Mode of action of mycobacterial fractions in anti-tumor immunity: Preliminary evidence for a direct nonspecific stimulatory effect of MER on immunologically reactive cells. Ann. N. Y. Acad. Sci. 276: 536-549, 1976.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01-CM-06850-07 PO

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

DNA and RNA Tumor Viruses: Molecular Mechanisms of Infection and Carcinogenesis

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	A.S. Levine, Head, Infectious Disease Sect.	PO C
OTHER:	C.T. Patch, S.K. Chattopadhyay, I.T. Magrath, P.A. Pizzo, G.W. Jay: Senior Investigators, Infect. Dis. Sect.	PO C
	B.S. Kramer, C.B. Freeman,: Clinical Associates	PO C
	E. Jaffe, Senior Investigator; O. Alabaster, Expert; R. Braylan, Visiting Scientist; N.A. Young, Head, Viral Oncology & Molec. Biol.	LP C
	A.M. Lewis, Senior Investigator; W.P. Rowe, Chief, Lab. of Viral Diseases; J.W. Hartley, Senior Investigator	LVD I
	P. Gerber, Chief, Viral Genetics	DV BB
	M. Lippman, J. Peng; Senior Investigators	M C
	R.J. Biggar, F.Nkrumah, Burkitt's Tumor Project	Accra, Ghana
	N. Davidson, Professor	CAL Tech.
	F.T. Jay, Visiting Fellow	LEP A

COOPERATING UNITS (if any)

Laboratory of Viral Diseases (NIAID); Biochemistry Department (CAL Tech.): Laboratory of Experimental Pathology (NIAID): Laboratory of Pathology (C); Division of Virology (BB); Medicine Branch (C); Burkitt's Tumor Project (Accra, Ghana)

LAB/BRANCH

Pediatric Oncology Branch

SECTION

Infectious Disease Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20014

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SUMMARY OF WORK (200 words or less - underline keywords)

We are studying the genetic organization and expression of SV40 and adenovirus 2 by an analysis of the SV40 and Ad2 genomes contained within Ad2-SV40 hybrid virus DNA molecules. The hybrid viruses are also employed as models for understanding the behavior of integrated genomes. We are also analyzing the behavior of SV40 and Ad2-transformed or lytically infected cells as a function of the molecular conformation of viral nucleic acids within these cells, and as a function of events which may control (or be controlled by) viral replication, transcription, and translation. Secondly, we are analyzing the state and expression of the endogenous AKR murine leukemia virus genome in mice with varying expression of leukemia and virus production. A further objective is to explore the evolution, transmission, and relation to leukemogenesis of various murine RNA viruses. Finally, this laboratory is concerned with production and purification of Epstein-Barr virus DNA and the use of this reagent to explore molecular mechanisms of infection and oncogenicity associated with this human herpes virus. Studies of cell lines from human lymphomas include surface markers, karyotypes, presence of and infectibility by EBV, growth characteristics, and morphology. New EBV strains from African Burkitt's (true tumor strains?) are studied.

Project Description/

Objectives:

1) To study the genetic organization and expression of Simian Virus 40 (SV40) and human Adenovirus 2 (Ad2) by an analysis of the varying segments of the SV40 and Ad2 genomes contained within Ad2-SV40 hybrid virus DNA molecules. Such studies employ biologic, biochemical, biophysical, and electron microscopic techniques to achieve genetic maps of these animal tumor viruses. The hybrid viruses are also employed as models for understanding the behavior of integrated genomes, the phenomenon of integration appearing central to the molecular mechanism of viral oncogenesis.

2) To analyze the behavior of SV40 and Ad2-transformed or lytically infected cells as a function of the molecular conformation of viral nucleic acids within these cells, and as a function of events which may control (or be controlled by) viral replication, transcription, translation and maturation.

3) To improve the production and purification of Epstein-Barr virus (EBV) DNA and to use the reagent to explore the molecular mechanisms of infection and oncogenicity associated with this human herpes virus; to more precisely identify the probable cell of origin of Burkitt's lymphoma; to attempt to provide better diagnostic criteria of lymphomas by providing data at a functional level which relates to specific lymphoma sub-types; to compare EBV genome-positive cell lines with EBV genome-negative lines and to determine whether differences relate simply to the presence of the genome, or reflect a difference between cells which can be infected by EBV in vivo and cells which cannot; to determine the relevance of different strains of EBV to the range of EBV-associated diseases and to associate biological differences in virus strains with biochemical differences.

4) To enhance our understanding of the natural history of murine leukemia by analyzing the state and expression of the endogenous AKR murine leukemia virus genome in rodents with high-, low-, or no incidence of leukemia. A further objective is to explore the evolution, transmission and relation to leukemogenesis of this virus, using cells from a variety of inbred rodents and related species indigenous to widespread parts of the world.

Methods Employed:

Procedures for the isolation of highly purified viral and cell macromolecules are continuously improved and new techniques for hybridization of viral and cellular nucleic acids are developed. A variety of techniques which permit quantitative comparison of nucleotide sequences are in use. This laboratory routinely employs equilibrium density centrifugation, analytical ultracentrifugation, electron microscopic heteroduplex mapping, enzymatic fragmentation and strand separation of viral DNA, gel electrophoresis, hydroxyapatite chromatography, and the in vitro synthesis of viral DNA probes. Nucleotide sequencing of specific viral RNA transcripts is available. In vitro protein synthesis using cell-free systems is undertaken. An extensive virus purification and tissue culture facility is maintained; plaque assays and CF or IF tests for viral-specific antigens are performed. Radiolabelled proteins are analyzed via autoradiography or fluorography; fingerprint analysis of the tryptic peptides of purified proteins is performed by a two-dimensional procedure involving high-voltage electrophoresis and thin-layer chromatography. Mouse breeding experiments are performed in association with studies on the genetics of RNA tumor viruses. Immunological methods include study of surface immunoglobulins and rosette-forming cells utilizing a variety of reagents including those made from sheep erythrocytes, antibody and complement, and also from fluoresceinated salmonella typhi. Reagents made from purified complement components represent an essential part of the methodology. Immunofluorescent assay is used for EBV virus infection of genome-negative cell lines, and flow microfluorimetry for characterization of distribution of cell size, DNA content, and protein content within the cell population.

Major Findings:

A. Studies of Naturally Occuring Murine Leukemia Viruses

Utilizing a [^{3}H] viral DNA probe synthesized from naturally occuring AKR murine leukemia virus RNA, we have established that a good correlation exists between the completeness of the viral sequences in the host genome, the number of copies of the viral-specific sequences, leukemogenesis, and the production of virus by various mouse strains. Furthermore, we have demonstrated that a major virus-inducing locus, Akv-1, is a viral structural gene. We have now extended this study by determining the number of copies of the AKR virus genome integrated into the cellular DNA after exogenous infection of NIH mouse, AKR mouse, and rat cells in tissue culture. NIH mouse cells, which lack a portion of the viral genome (referred to as Gross-AKR specific sequences), incorporated three to four copies of these sequences per haploid genome. AKR cells, in which the Gross-AKR specific sequences are already present as three to four copies per haploid genome, did not show any distinct change in copy number after infection. Rat cells, which lack DNA sequences homologous to murine leukemia virus, incorporated one copy of the viral genome per haploid genome. It is inferred that the presence of viral sequences may affect the efficiency of integration of exogenous provirus, and that there may be a limit to the number of copies that can be inserted.

Naturally occurring murine leukemia viruses, all of which are readily isolated from mouse cells, can now be separated into three groups on the basis of their host range, interference specificity, and virus neutralization. These groups are the (a) Ecotropic (or mouse-tropic) MuLV strains which can exogenously infect mouse cells but not cells of other species; (b) Xenotropic MuLV which cannot infect mouse cells exogenously, but can infect cells of certain heterologous species, e.g., rabbit, mink, and human; and (c) Amphotropic MuLV which exogenously infects mouse cells as well as cells from heterologous species. Utilizing a single-stranded [^{3}H] DNA probe synthesized from two xenotropic (NZB-IU-1 and BALB-1U-1) viruses and an amphotropic (1504-A) virus, we examined several inbred and wild mouse strains. Our analysis has shown that the complete genomes of all of these viruses are present in multiple copies in the DNA of all mouse strains so far tested. By hybridization of [^{3}H] DNA probes and RNAs from either purified virions or from producer cells, we have been able to characterize biochemically several naturally occurring ecotropic, xenotropic and amphotropic viruses. In general, all of these viruses share a common group of sequences; in addition, they do possess a unique group of sequences. In one ecotropic (AKR) and one amphotropic (1504-A) virus, these unique sequences represent 20% of the total viral genome. By nucleic acid hybridization techniques, including hydroxyapatite chromatography, we have been able to purify and characterize the unique sequences from ecotropic-AKR and amphotropic 1504-A.

B. Mechanism of IUdR Activation of Virus

Halogenated pyrimidines (e.g., IUdR) activate C-type virus production from normal cells of a wide variety of animals in tissue culture. The pyrimidines become incorporated into the DNA of these cells, but the mechanism by which they activate virus is not known. In a normal (non-producer) cell line derived from AKR embryos, about 30-40 per cent of the viral sequences are present in cellular RNA. However, after treatment with IUdR, 100 per cent of the viral-specific sequences are expressed in cell RNA. To understand the mechanism of this IUdR effect, we have attempted to determine whether viral-specific sequences are newly and exclusively transcribed after IUdR treatment, or whether this drug increases the overall transcription of cellular DNA with integrated viral sequences comprising one part. Our hybridization experiments with [^{3}H] unique mouse cellular DNA and RNA indicate that IUdR increases the overall transcription of mouse cellular DNA, i.e., sequences that had not been transcribed now are. We could not however detect any alteration of the transcription pattern of the sequences that were being transcribed before IUdR treatment, except for their rate of synthesis and/or accumulation in the cell.

C. SV40 - specific Ribosome - Binding Proteins Induced by Ad2$^+$ND$_2$

We have studied the intracellular distribution of the two SV40-specific proteins, with apparent molecular weights of 56,000 and 42,000, detectable in human KB cells infected by a nondefective adenovirus 2-simian virus 40 hybrids, Ad2$^+$ND$_2$. After a twenty-minute pulse of [^{35}S]methionine, about two-thirds of the newly-synthesized 56K protein and one-third of the 42K protein were found to be localized on the plasma membrane. The remainder of each protein was found in the cytoplasm, while the nuclear fraction was virtually free of either component. A significant portion of both proteins present in the cytoplasmic fraction was complexed to the 40S ribosomal subunits, and was not removed by treatment with 0.5 M KCl. Moreover, the portion that was found free in the cytoplasm could bind preferentially and quantitatively to purified 40S ribosomes *in vitro*, leading us to propose that these SV40 proteins may act as translational control elements in the cell.

D. TSTA is Virus-coded

We have shown that mice preimmunized with the SV40-specific proteins synthesized by the Ad2-SV40 hybrids (the 28K protein from Ad2^{+}ND$_1$-infected cells, and the 42K and 56K proteins from Ad2^{+}ND$_2$-infected cells) have the ability to reject tumor formation upon subsequent challenge by SV40 tumor cells. Since these SV40-specific proteins have previously been shown to be coded by the SV40 genome, our findings prove definitively that the tumor-specific transplantation antigen (TSTA) is viral coded. Furthermore, our results show that the SV40 fragment present in Ad2^{+}ND$_1$ is sufficient to code for the antigenic determinant responsible for inducing transplantation rejection.

E. Content and Expression of Integrated Viral DNA in Hamster Cells Transformed by Nondefective Adenovirus Type 2-Simian Virus 40 Hybrid Viruses

Hamster cells transformed by adenovirus 2 (Ad2) and five nondefective Ad2-simian virus 40 (SV40) hybrid viruses are all of the Ad2-transformed phenotype. All lines accumulate Ad2 RNA and Ad2 T antigen; two hybrid-transformed lines accumulate SV40 RNA, but only one contains detectable amounts of SV40 antigens. We examined selected lines from this group of transformed hamster cells for Ad2 DNA content and viral RNA expression by using hydroxyapatite chromatography and separated strands of labeled viral DNA as probes. All lines studied contain 1 to 2 Ad2 genome equivalents per haploid equivalent of cell DNA. The expression of Ad2 RNA exhibits two distinct patterns. In one pattern, transcription of the heavy strand of Ad2 DNA is less extensive than that of the light strand, whereas in the second pattern of Ad2 RNA expression the extent of heavy-strand transcription is greatly increased. In the two lines containing SV40 RNA, the entire SV40 (-) strand is transcribed; the (+) strand is not expressed in these cells. These patterns of viral RNA expression suggest the possibility that host promoters may play a role in regulating transcription of integrated viral DNA.

F. Transcription of Integrated Viral DNA in Hamster Embryo Cells Transformed by Ad2, Ad2^{+}ND2 and Ad2^{+}ND^{4}.

It has been suggested that the viral RNA sequences which accumulate in the cytoplasm of Ad2-transformed rat cells are transcribed only from early Ad2 templates and that late Ad2-lytic cytoplasmic RNA is derived by processing of complete transcripts (of both strands) which accumulate in the nucleus. In the present study, 13 embryo cell lines transformed by Ad2 or nondefective Ad2-SV40 hybrids have been examined for DNA content and RNA expression, using the separated light (L) and heavy (H) strands of Ad2 DNA as probes. The values for L/H and L+H obtained by hybridizing Ad2 DNA with cytoplasmic RNA from cells transformed by Ad2, Ad2^{+}ND2, and Ad2^{+}ND4 agree well with the expected values for early Ad2-lytic cytoplasmic RNA. While the L+H values for nuclear RNA are similar to the L+H values for cytoplasmic RNA in the case of Ad2-transformed cells, the corresponding values for cells transformed by Ad2^{+}ND$_2$ and Ad2^{+}ND$_4$ differ by a factor of two. Our data suggest that the RNA which accumulates in the cytoplasm of cells transformed by Ad2, $\text{Ad2}^+\text{ND}_2$, and $\text{Ad2}^+\text{ND}_4$ is derived by processing from more extensive nuclear transcripts; the cytoplasmic RNA may be comprised only of transcripts of the early viral templates. Furthermore, different promoters may regulate transcription of viral sequences in Ad2 transformed cells as contrasted with cells transformed by $\text{Ad2}^+\text{ND}_2$ and $\text{Ad2}^+\text{ND}_4$. These data further suggest that RNA processing is under viral control in all cases. To confirm these mechanisms, we will examine RNA using endonuclease cleavage fragments of Ad2 DNA as hybridization probes. The amount of viral DNA detected in each cell line is sufficient to provide templates for the RNA detected, and it would appear in some cases that the nuclear RNA contains transcripts of most of the integrated viral DNA. In most cases, less than half of the viral genome appears to be present in transformed cell DNA. Since the early templates in Ad2 are located at the ends of the viral genome, these data suggest that the viral DNA deleted during integration must be removed from the middle of the genome if the early templates remain integrated as required by the model presented above. This appears to be the case in some Ad2 transformed rat cells; analysis with Ad2 DNA fragments will be required to determine whether this situation exists in the case of transformed hamster embryo cells.

G. Determination of the Number of Copies and the Proportion of Viral Genomes Integrated in Transformed Cellular DNA

An equation has been derived that relates the maximum fraction (F) of a single-stranded DNA probe that hybridizes with a given quantity of cellular DNA to the number of copies (N) and the proportion (X) of the probe contained in the cellular DNA: $1/F = 1/X + (1/NX)\,R$ (equation 1), where: $R = 2\,C_0^p M_c / C_0^c M_p$ and: C_0^p and C_0^c are the initial concentrations of probe and cellular DNA, respectively; M_p and M_c are the molecular weights of the probe and cellular DNA, respectively. The validity of equation 1 has been verified by hybridizing single-stranded radioactive SV40 DNA with double-stranded DNA from the nondefective Ad2-SV40 hybrid virus $\text{Ad2}^+\text{ND}_4$ (which has a well-defined segment of integrated SV40 DNA) or mixtures of $\text{Ad2}^+\text{ND}_4$ and SV40 DNAs. The data from these experiments, when analyzed by equation 1, yield values of X and N that are in good agreement with the expected values. It should be noted that equation 1 can be used to analyze hybridization experiments employing any single-stranded DNA probe derived either from intact viral DNA or from endonuclease cleavage fragments of viral DNA. Equation 1 has also been used to analyze data obtained by hybridizing single-stranded Ad2 and SV40 DNA with cellular DNA prepared from cells transformed by Ad2, $\text{Ad2}^+\text{ND}_2$, and $\text{Ad2}^+\text{ND}_4$. The results indicated that cells transformed by these viruses contain various proportions of the viral genome ranging from 2/3 to intact, and these genomes are present in 1-3 copies, in agreement with results obtained by RNA-DNA hybridization and DNA-DNA reassociation kinetics.

H. Effect of IUdR Incorporation on Viral Transcription

Culturing mouse cells with IUdR activates propagation of murine leukemia virus. These cells are also stimulated to increased RNA synthesis by IUdR. However, the mechanism(s) by which these phenomena are mediated is unknown. Promoter regions may be sensitive to small perturbations such as replacement of thymine residues with IU. SV40 transcription *in vivo* and *in vitro* has been extensively studied and perturbations introduced by IUdR should be easily recognized; identification of the perturbation should provide insight into the general mechanism of IUdR action. SV40 in which the DNA is extensively labeled with IUdR can be prepared by infecting Vero cells in the presence of IUdR (20 μg/ml). The viral DNA in these preparations is mainly in the superhelical form as determined by isopycnic CsCl gradients (with ethidium bromide) and by sedimentation in alkaline sucrose. IUdR-labeled SV40 DNA is 0.072 g/cc more dense than unlabeled SV40 DNA, from which it is estimated that at least one-third of the thymine residues are replaced by IU. Unlabeled virus is about 30 times more infectious than IUdR labeled SV40; similarly the ability of labeled SV40 to induce T ag is significantly reduced.

I. Synthesis and Processing of the Precursor to the Major Core Protein of Adenovirus Type 2

An isopycnic Metrizamide-detergent gradient system was developed in which the newly synthesized precursor (polypeptide P-VII) to the major core protein of adenovirus type 2 (polypeptide VII) was confined to a spectrum of complexes with densities equal to or higher than that of adenovirions. The majority of the newly synthesized P-VII was, at the beginning of the logarithmic period of virus production, present as an entity of protein density. This pool of P-VII was efficiently depleted. P-VII was also associated with high-molecular-weight structures of intermediate density, sharing some properties with empty capsids or incomplete particles. The transfer of P-VII from the intermediate-density region was not quantitative, and only particles of true virion density region subsequently contained polypeptide VII. No structures equivalent to the core structure of disrupted virions or identical to incomplete particles were detected in this system. A temperature-dependent transition of radioactivity from polypeptide P-VII into polypeptide VII was also detectable after in vitro incubation of P-VII-containing complexes. Addition of Ad2-infected cell extracts was required for processing of complexes derived from regions of protein density, whereas P-VII was processed spontaneously upon incubation in complexes of virion density.

J. Induction of Complement Receptors in Lymphoma Cells

Two EBV-genome negative lymphoma cell lines can be induced by means of xanthine derivatives to increase dramatically their expression of surface complement receptors. Normally these cells express a very low percentage of receptors detectable by erythrocyte-antibody-complement complexes (EAC') - e.g., 0-10%; when incubated with theophylline for 24 hrs, some 30-70% of cells express complement receptors. Induction of receptors does not occur in the presence of actinomycin D and puromycin, and hence appears to be an active synthetic process dependent upon RNA and protein synthesis. Dibuteryl cyclic AMP does not induce receptors. The receptors induced differ in several ways from the known complement receptors of normal lymphocytes and EBV genome-positive cell lines. Thus they are detected by a fluoresceinated, complement-treated bacterial preparation preferably at 37°C, whereas complement receptors of Raji cells (C3b and C3d) can be detected equally well at 0° and 37°C. Specific reagents (e.g., fluoresceinated C3b, and EAC 1-3b and EAC 1-3d) do not react with the induced receptors.

K. EBV Replication

We have shown by immunofluorescence, electron microscopy, and quantitation of viral DNA that virus replication can be induced by corticosteroids. Factors which affect the induction efficiency include spontaneous fluctuations in virus production by a given cell line, differences in virus production and susceptibility to steroid-induced growth inhibition in different cell lines, the type, concentration and day of addition of steroid, and the temperature of incubation. In one HR1 cell line (provided by Dr. B. Hamper), the concentration of dexamethasone which induces virus replication far exceeds the concentration which will saturate steroid receptors.

L. New EBV Strain

A new virus strain - AG876- has been isolated from a recently derived tumor cell line obtained from a patient with Burkitt's lymphoma in Ghana. Biological studies carried out with Dr. P. Gerber have shown that this virus is a potent transforming agent for cord blood lymphocytes, but will not induce early antigen in Raji cells. Experiments are currently in progress to determine the oncogenicity of this virus strain in marmosets. AG876 DNA has been purified and its reassociation kinetics examined in the presence of DNAs obtained from the parent cells of the prototype "infectious," non-transforming (HR1) and transforming viruses (B95-8). AG876 contains at least 95% of the sequences present in HR1 and B95-8. Further, reassociation kinetics of HR1 in the presence of AG876 DNA indicate HR1 virus does not contain additional sequences not present in AG876. The biological differences in these strains must, therefore, relate to subtle differences in the arrangement of nucleotide sequences, or small differences in parts of the viral genomes which cannot be detected by hybridization experiments.

Significance to Biomedical Research and the Program of the Institute:

In studies on the mouse-tropic, xenotropic, and amphotropic murine leukemia viruses, our objective is to correlate specific leukemia virus sequences with specific biological events, especially leukemogenesis. Comparative study of the genetic information specified by different murine leukemia virus sequences in different strains of mice is now feasible, and we believe that it will be possible to determine the evolutionary origin and transmission of murine leukemia virus infection. Our studies during the past year have explained why only some mice produce virus and develop leukemia even though all mice may contain MLV antigens. Moreover, it has been established that a major mouse chromosomal locus for the production of MLV is in fact composed of MLV nucleotide sequences, and is not merely a host locus which permits the expression of remote viral functions. It should be emphasized that these studies concern a known naturally occuring animal leukemia virus within the context of known and controlled host genetics. We believe that these studies will guide the framing of testable hypotheses in human leukemia, wherein a proven leukemia virus has not been characterized and where the genetics of the disease are not known and cannot be controlled.

Our studies of Ad2-SV40 hybrids have made a significant contribution to knowledge of genetic organization and expression of the two tumor viruses during lytic infection and oncogenesis. Moreover, these hybrid virus studies offer insight into the state and expression of integrated heterologous DNA in general and must be considered in planning the type of "genetic engineering" experiments possible with recombinant DNA technology.

Whether a specific viral infection will lead to virus production or to cell transformation depends entirely upon the interaction between the incoming virus and the host cell involved. At the molecular level, such interactions may occur between virus-specific macromolecules and macromolecules specified by the host. Our studies with Ad2 and SV40-antigens are directed toward an understanding of these interactions; our hope is to provide a better understanding of the mechanisms of viral infection and oncogenesis.

EBV is closely associated with at least three human diseases - infectious mononucleosis, African Burkitt's lymphoma and nasopharyngeal carcinoma. As such it represents a potential human tumor virus, and clarification of its role in the diseases with which it is associated is of paramount importance. Studies on the induction of EBV with corticosteroids may clarify the relationship of EBV to the host cell since they suggest that the viral genome may be influenced by mechanisms which regulate the transcription of unique genes in differentiated cells. Such information is pertinent to an understanding of the association between latent virus infection and oncogenesis - both of which are important characteristics of the herpes virus group as a whole. Studies of different strains of EBV will help to determine whether or not differences in virus properties are relevant to the association of EBV with sub-clinical, benign and malignant diseases. Finally, our studies have revealed the imprecision of reagents currently used to detect complement receptors on normal and malignant cells. We feel that use of better defined reagents will reveal differences in the surface receptors of lymphoma cells which were previously not realized, and which may be important in more precisely defining the counterpart normal cell. Hopefully these findings will provide an improvement in the classification of lymphomas

Proposed Course:

We shall continue to employ various biochemical and biological techniques to distinguish between the viral sequences specified by different endogenous murine leukemia viruses in the DNA of mice with differing expression of MLV information. DNA from other rodent species will be tested for homology with various MLV DNA probes, and these studies should speak to the evolutionary origin, transmission, completeness and expression of the MLV genome. We shall attempt to delineate further the molecular mechanism of viral activation by halogenated pyrimidines, and will also extend these studies with other activating agents.

Studies on the nondefective Ad2-SV40 hybrid viruses will emphasize the kinetics of SV40 transcription early and late in infection, and the perturbations in transcription displayed by IUdR-containing SV40 DNA. A new project involves SV40 transformation of human cells; various human cell lines differ in transformability by SV40 as determined by focus formation and induction of T ag. Attempts will be made to correlate these properties with the ability of the various human cell lines to absorb and express SV40. SV40-transformed lines will be derived and the amount and expression of integrated viral DNA determined. Of particular interest will be cell lines derived from individuals within families that are highly susceptibile to leukemia: can mechanisms be found that relate the increased SV40 transformability in such families to their leukemogenic process? Finally, with respect to SV40, this virus has been shown to induce two immunological activities, T antigen and U antigen, that are detectable by indirect immunofluorescence or complement fixation. However, it is not clear whether the two activities represent two distinct macromolecules or two antigenic determinants present within a single macromolecule. Since some of the Ad2-SV40 hybrids induce only U but not T antigen, it should be possible to differentiate between the two possibilities. By radioimmunoprecipitation, we will study the antigenic relationships between each of the SV40-specific proteins and attempt to identify the antigenic determinants responsible for each of the two immunological activities. Unlike T antigen which is detectable by in vitro immunological tests, TSTA is detected separately by in vivo methods. Since the early region of the SV40 genome contains barely enough genetic information to code for T antigen, a protein of approximately 90,000 molecular weight, it is not clear whether this same protein also functions as TSTA. We will use an anti-T serum to specifically and quantitatively remove the 90K molecular weight T antigen from a cell extract, and try to determine whether the T antigen-depleted extract has also lost its ability to protect mice against tumor induction by syngeneic SV40 tumor cells. It has been suggested that SV40 T antigen may have a pleiotropic effect on various cellular functions in viral transformation. Since each of the Ad2-SV40 hybrid viruses has been shown to synthesize SV40-specific polypeptides of different molecular weights but with overlapping amino acid sequences, we shall attempt to use the hybrids to map the "active sites" of various cellular functions and the various properties of T antigen (which appears to be coded by the entire early region of the SV40 genome).

In our experiments with Epstein-Barr virus, we shall continue to optimize the system for production and purification of this virus and its DNA: we shall measure the genetic relatedness of EBV strains of different origin, study the relationship between EBV DNA and host cell DNA, and try to relate our findings to EBV-associated human disease. Further studies are planned of the mechanism of steroid induction of EBV - e.g., the role of steroid receptors. It is hoped that more virus strains can be obtained, and comparisons made with the viral sequences present in cell lines derived from patients with infectious mononucleosis, and from throat-washing induced cell lines obtained from patients in different parts of the world. These studies will be extended by utilizing restriction endonuclease fragmentation of the viral genomes. We shall also continue in our efforts to determine whether the induction of receptors on lymphoma cells is brought about by direct switching on of a unique gene, representing a differentiation step, and whether or not cyclic AMP is involved. In addition we shall attempt to provide reagents which give a much greater degree of precision in identification of complement receptors. These studies will be extended by examining other functional characteristics of tumor cells or their derived lines and correlating such a functional classification of lymphoma with morphology and clinical course.

Publications:

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2. Chattopadhyay, S.K., Levine, A.S. and Rowe, W.P.: Biochemical detection of murine leukemia virus genomes in mouse chromosomes. Proceedings of the Symposium on Chromosomes in Evolution, The Nucleus, in press.

3. Chattopadhyay, S.K., Levine, A.S., and Rowe, W.P.: Presence of naturally occurring murine leukemia viral sequences in mouse chromosomes and their correlation with murine leukemia. Proceedings of the Symposium on the Molecular Basis of Host-Virus Interaction, Science Press (Princeton), in press.

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8. Jay, G., Jay, G.T., Friedman, R.M., and Levine, A.S.: SV40-specific ribosome-binding proteins induced by a non-defective adenovirus 2-SV40 hybrid. J. Virol., in press.

9. Lowy, D.R., Chattopadhyay, S.K. and Teich, N.M.: The chromosomal localization of an endogenous murine leukemia viral genome in the AKR mouse. In Yuhus, J.M., Tennant, R.W., and Regan, J.D. (Ed.): Biology of Radiation Carcinogenesis. New York, Raven Press, 1976, pp. 195-205.

10. Magrath, I.T., Pizzo, P.A., and Levine, A.S.: Induction of Epstein-Barr virus replication by corticosteroids, in press.

11. Patch, C.T., Levine, A.S., and Lewis, A.M.: Defective and nondefective adenovirus 2-SV40 hybrids. In Wagner, R. (Ed.): Comprehensive Virology, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01-CM-06860-01 PO

PERIOD COVERED
7/1/76 - 9/30/77

TITLE OF PROJECT (80 characters or less)

Clinical and Molecular Pharmacology; CNS Pharmacokinetics

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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SECTION

INSTITUTE AND LOCATION
NCI. NIH. Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
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SUMMARY OF WORK (200 words or less - underline keywords)

This project concerns clinical, preclinical, and biophysical studies of anticancer drugs. Clinically useful assays for citrovorum factor and 8-azaguanine in body fluids have been developed. The assay for 8-azaguanine has been used to study the pharmacokinetics of intraventricular administration in monkeys. Both assays are being applied to studies in man. Preclinical studies of systemic and cerebrospinal fluid pharmacokinetics in monkeys and dogs have been initiated on a variety of antineoplastic drugs. These studies are also being used to explore potentially useful interactions of chemotherapeutic agents with other drugs. An electric dichroism apparatus has been constructed for use in characterizing interactions of small molecules (carcinogens and anticarcinogens) with macromolecules of biological importance. Such measurements may be helpful in evaluating analogues of known and newly proposed drugs. The results may also permit the use of small molecules as probes of subcellular structure. We are also investigating new treatments for patients with CNS leukemia, based on an innovative primate model which allows direct access to the ventricular cerebrospinal fluid, and thus facilitates investigation of CSF pharmacokinetics.

Objectives:

1. To develop and apply clinically useful assays for chemotherapeutic agents.
2. To characterize the pharmacokinetics of chemotherapeutic agents and related compounds and to thereby enhance their utility.
3. To utilize physical-chemical techniques to characterize the complexes of small molecules, both antineoplastic agents and carcinogens, with acids and other biopolymers.
4. To utilize the information gained in #3 to facilitate evaluation of analogs of known chemotherapeutic agents and newly synthesized compounds.
5. To utilize agents characterized in #3 and #4 as probes of macromolecular and subcellular structure in neoplastic cells.
6. To improve the therapy of meningeal leukemia by detailed investigation of cerebrospinal fluid pharmacokinetics in an animal model.
7. To develop an animal model of methotrexate leukoencephalopathy with the aim of prevention and treatment of this syndrome.
8. To evaluate the pharmacokinetics of chemotherapeutic agents used to treat central nervous system malignancies.

Major Findings and Methods:

A. An assay system for citrovorum factor (N-5-formyl tetrahydrofolic acid) has been developed using a bacterially derived cell-free synthesizing system with appropriately radiolabeled components. The system appears to be sensitive to approximately 10^{-6} molar citrovorum factor. We have developed appropriate control curves for buffer and human plasma systems and are presently testing the assay on patients receiving citrovorum factor.

B. 8-azaguanine is a compound that has been employed in the treatment of malignant gliomas in humans with some suggestion of success. We have developed a spectroscopic assay for 8-azaguanine in cerebrospinal fluid. The assay system is presently sensitive to approximately 10^{-5} molar. We have characterized the pharmacokinetics of 8-azaguanine given intrathecally in monkeys over the first 13 hours subsequent to infusion. In collaboration with Dr. Ayoub K. Ommaya, we plan to measure the pharmacokinetics of this compound in patients receiving the drug for malignant gliomas as part of a protocol presently being undertaken.

C. We have characterized the effect of systemic probenecid on the intrathecal administration of methotrexate in the dog. We have shown that systemic probenecid inhibits the transfer of methotrexate from cerebrospinal fluid to plasma (i.e., the excretion of methotrexate from CSF following intrathecal administration). We are attempting to extend these observations to the monkey and to characterize both the systemic and cerebrospinal fluid pharmacokinetics of methotrexate in the monkey following probenecid administration systemically, utilizing both I.V. bolus and intrathecal administration. Similar data are presently being obtained on methotrexate pharmacokinetics following administration of vincristine. The pharmacokinetics of a variety of other chemotherapeutic agents (e.g., cis-platinum, aminopterin) are presently being pursued in the monkey model.

D. We have completed construction of an electric dichroism apparatus for use in characterizing the geometry of complexes of small molecules, such as antineoplastic agents, with nucleic acids and other biological macromolecules of interest. The system has an online computer for rapid data analysis. We intend to apply this system to studying a series of ethidium bromide derivatives and their interaction with nucleic acids, as well as to a series of carcinogens which are derivatives of benzpyrene. Other compounds of interest include psoralens which are being studied in collaboration with Dr. John Hearst of the University of California at Berkeley.

E. Dr. Poplack and his co-workers have recently developed an experimental model that enables repetitive sampling of cerebrospinal fluid over extended periods of time in unanesthetized Rhesus monkeys. This technique involves the subcutaneous implantation of an Ommaya reservoir with a silastic Pudenz catheter through the Foramen of Magendie into the fourth ventricle, without tissue damage or breakdown of the blood-brain barrier. The development of this model has allowed us to evaluate critically the CNS pharmacokinetics of a number of currently utilized and potential CNS antineoplastic agents. This model: (1) permits sterile access to cerebrospinal fluid without needle tract artifacts or long term immobilization; (2) provides mixing of injected drugs with lateral ventricular CSF; (3) enables spino-ventricular perfusion; (4) permits ventricular CSF sampling over extended periods; (5) enables sensitive monitoring of intraventricular pressure; and (6) provides pharmacokinetic data similar to that obtained in man. Our studies of methotrexate pharmacokinetics in the central nervous system have proven the validity of this model as a substitute for experimentation in the human situation. Human and primate data are strikingly similar, indicating that the kinetics (and presumably the mechanisms) of transport between the cerebrospinal fluid and plasma compartments are similar in both man and monkey. Since detailed pharmacokinetic analysis derived from this primate model can help predict the distribution kinetics of cytotoxic agents in man, the use of this system offers the opportunity to study both blood-brain and CSF-brain pharmacokinetics and also allows us to assess the neurotoxic effects of drugs used to treat central nervous system neoplasms. We have done extensive studies of methotrexate using this primate model: In addition to validating the utility of the model and its applicability to the human situation, we are examining new methods of administering methotrexate. We are also developing a primate model of methotrexate leukoencephalopathy. We have studied a group of animals, all of whom have been given biweekly intraventricular injections of methotrexate via the Ommaya reservoir. Half of these animals also received

1250 rads in a nominal single dose of cranial irradiation (a dose analogous to 3500 fractionated rads, which is in the therapeutic range for humans). It now appears that animals who have received both irradiation and methotrexate are neurotoxic. Evaluation of their neurological status by computerized tomographic scanning and pathological examination should indicate whether we have been successful in developing an appropriate model.

Significance to Biomedical Research and the Program of the Institute:

The utility of a biochemical assay for citrovorum factor in monitoring patients receiving either high-dose methotrexate or sustained methotrexate infusions is of clear interest in the clinical management of these patients. Relatively little is known about the pharmacokinetics of citrovorum factor at the present time and attempts are under way, using radiolabeled citrovorum factor as well as this assay, to characterize these pharmacokinetics in man. A biochemical assay such as this would facilitate the use of such measurements on a routine basis in patients, and would allow relatively complete characterization of the kinetics of the administration of a folic acid antagonist such as methotrexate and a rescue agent such as citrovorum factor. The assay system for 8-azaguanine will permit characterization of the pharmacokinetics of this agent in patients and thereby enhance its utility. The results on the interaction of methotrexate and probenecid, if they can be extended to man, would lead to the possibility of administering systemic methotrexate for central nervous system treatment by preventing the egress of methotrexate from the CSF after probenecid pretreatment. These results also raise the possibility of enhanced exposure to methotrexate in patients who are receiving other drugs, with resulting increased risk of neurotoxicity in patients who are receiving other drugs. The possibility of taking therapeutic advantage of interactions between chemotherapeutic agents and other pharmaceuticals is also suggested by studies of this kind. Basic biophysical studies on the interaction of chemotherapeutic agents with biologically important macromolecules enhance the utility of these agents clinically, and improve their usefulness in the solution of biochemical problems related to the structure of macromolecules and to the basic process of neoplasia. Finally, an animal model which allows repetitive sampling of ventricular cerebrospinal fluid constitutes a significant development which will now allow meaningful, accurate pharmacokinetic analysis of CNS antineoplastic agents.

Proposed Course:

It is our intention to utilize those assay systems which appear to be ready for trials in man and to begin to gather data on patients. We intend to extend the studies on the interaction of methotrexate and probenecid, as well as other pharmacokinetic studies, to model systems more closely resembling clinical situations and to subsequently apply them to patients. Basic biophysical studies on complexes of chemotherapeutic agents and carcinogens with nucleic acids may, in the case of the psoralens, permit a more refined view of cell kinetics. In the cases of the other series of compounds, they will permit solution of certain basic biochemical problems regarding macromolecular structure. Use of the primate model which we have employed to study cerebrospinal fluid pharmacokinetics will be expanded in an effort to increase the applicability of this important model system. The overall program is designed to permit a more basic understanding of the mechanisms of action of chemotherapeutic agents, a fuller view of their pharmacokinetics, and hopefully, to improve our ability to apply chemotherapeutic agents rationally in clinical situations.

Publications:

1. Bleyer, W.A., Poplack, D.G., Simon, R.M.: Concentration X time methotrexate via a subcutaneous reservoir: a less toxic regimen for intraventricular chemotherapy of central nervous system neoplasms. In press.

2. Glaubiger, D.: Binding of ligands to deoxy-ribonucleic acid. In Selegny, E. (Ed.): IX Annual Symposium on Optically Active Polymers. In press.

3. Glaubiger, D. and Charney, E.: On the orientation of the phenoxazone ring in actinomycin D-DNA complexes, in press.

4. Poplack, D.G., Bleyer, W.A., Wood, J.H., Kostolich, M., Savitch, J.A., and Ommaya, A.K.: A primate model for study of methotrexate pharmacokinetics in the central nervous system. Cancer Research, in press.

5. Ramu, A., Glaubiger, D., Ramu, N.P., Eldridge, N. and Blaschke, N.: Probenecid inhibition of methotrexate excretion from cerebrospinal fluid in dogs, in press.

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7. Wood, J.H., Poplack, D.G., Bleyer, W.A., Ommaya, A.K.: Primate model for the chronic study of intraventricularly or intrathecally-administered drugs, and intracranial pressure. Science 195: 499-501, 1977.

8. Wood, J.H., Poplack, D.G., Flor, W.I., Gunby, E.N., and Ommaya, A.K.: Chronic ventricular cerebrospinal fluid sampling, drug injection, and pressure monitoring using a subcutaneous reservoir in the monkey. Neurosurgery, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01-CM-06870-01 PO

PERIOD COVERED
7/1/76 - 9/30/77

TITLE OF PROJECT (80 characters or less)

Regulation of Differentiation and Cell Division in Normal and Leukemic Hematopoietic cells

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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	A. Nienhuis, Chief, Clinical Hematology Branch	NHLBI
	F. Anderson, Chief, Molecular Hematology Laboratory	NHLBI
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	R. Ungerleider, Clinical Associate	PO C

COOPERATING UNITS (if any)

Medical School, Yale University; Clinical Hematology Branch, NHLBI; Molecular Hematology Laboratory, NHLBI

LAB/BRANCH
Pediatric Oncology Branch

SECTION
Experimental Hematology Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
6	4	2

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The techniques of cell and molecular biology are being used to analyze mechanisms which control the expression of differentiated genes in normal and leukemic hematopoietic cells and regulate their differentiation and proliferation. Mutant erythroleukemia (MEL) cells will be fused with human erythroid and non-erythroid cells to generate hybrid cells containing human globin genes. The state of the human and mouse globin genes in these hybrid cells and the MEL parent cell will be studied during induction of gene transcription by testing the sensitivity of these genes to nuclease (DNase II) digestion during the induction process. Such hybrid cells containing human globin genes will also be used to determine if genetic elements controlling the expression of globin genes are on the same or different chromosomes as are the structural genes. Constitutive and inducible markers of myeloid, lymphoid, and erythroid hematopoietic cells will be studied in hybrids derived by fusion of leukemic cells exhibiting erythroid, myeloid and lymphoid phenotypes. The sensitivity of these hybrid cells to erythropoietin or colony-stimulating-factor will also be tested to analyze the genetic determinants which control proliferation of these cells.

Objectives:

1. To study the mechanisms regulating the expression of one marker of erythroid differentiation, globin gene expression, as a model for the regulation of expression of markers of myeloid, lymphoid and erythroid differentiation in normal and leukemic states in man.

2. To identify the human chromosomes on which the human globin structural genes reside. This information will be used to develop mutant mouse erythroleukemia cells into which the chromosomes bearing the human globin structural genes can be introduced and permanently retained. These cells will be used as test systems to study the regulation of expression of the human globin genes.

3. To study hybrid cells derived by fusion of leukemia cell lines which exhibit commitment to myeloid, erythroid and lymphoid differentiation, so as to clarify what genetic mechanism governs differentiation and proliferation of the normal hematopoietic cell and to identify how this mechanism may become altered in leukemia.

4. To determine the genetic pattern (co-dominance, dominance, or recessiveness) of responsiveness to known stimulators of proliferation of cells committed to erythroid (erythropoietin), myeloid (colony stimulating factor) and lymphoid differentiation (mitogens). These studies may clarify what genetic determinants are important in the ordered growth of hematopoietic cells and how these are altered in the leukemic state.

5. To study families in which a genetic predisposition exists to the development of leukemia in order to identify possible genetic mechanisms for the development of disordered maturation and proliferation of hematopoietic cells which result in this disease. Particular emphasis is placed on viral-cell interaction, and the changes in the behavior of surface antigens (such as HLA specificities) which may attend the emergence of hematological malignancies in man.

Methods Employed:

The activities of this laboratory represent an attempt to combine the techniques of cell biology (cell culture and cloning, chromosomal analysis by light and fluorescent microscopy, mutagenesis, isozymal analysis, radioisotopic analysis of enzyme activity and protein synthesis, cell fusion, and development of *in vitro* systems for study of gene expression) with those of molecular biology (molecular hybridization assays for the expression and presence of unique gene sequences, oligo dT chromatography of mRNA, hydroxylapatite chromotography of DNA, synthesis of cDNA corresponding to the globin mRNAs of man and mouse, density gradient ultracentrifugation, and gel electrophoretic separation of nucleic acids) in order to study mechanisms governing regulation of gene expression in mammalian cells. A tissue culture laboratory equipped with environmental control devices is available as well as a complete laboratory for the study of gene expression by molecular hybridization.

Major Findings:

1. Use of Cell Hybrids Derived by Fusion of Mouse Erythroleukemia Cells with Human Erythroid and Non-Erythroid Cells to Study the Expression of Globin Genes: We have demonstrated that introduction of human chromosomes from erythroid cells bearing the globin structural genes into mouse erythroleukemia cells (MEL) by cell fusion generates cell hybrids in which the co-expression of human and mouse globin genes is observed. In contrast, fusion of the MEL cell and human fibroblasts results in cells in which the failure of expression of the human globin genes as well as the loss of expression of the mouse globin genes has occurred at the level of transcription. Furthermore, dimethyl sulfoxide, which stimulates the transcription of the globin genes in the MEL cell but has no effect on the expression of human globin genes in the normal human erythroid cell, stimulates the expression of the human globin genes once they have been introduced into the MEL cell. Since the globin genes of different species on different chromosomes in the same cell respond to the same exogenous stimulus, some workers have concluded that the induction process involves diffusable regulatory substances. These hybrid cells therefore would appear to have been a good system for the study of the gene expression were it not for the loss of human chromosomes which occurs from these cells during continued culture *in vitro*.

2. Chromosomal Localization of the Human Alpha Globin Structural Genes: In order to generate continuously proliferating hybrid cells in which permanent retention of the human globin genes occurs, it was necessary to identify the chromosomal location of one or more of the human globin genes. This information, once obtained, would be of use in the interpretation of experiments designed to identify whether genetic elements regulating the expression of unique gene sequences are syntenic or asyntenic to the structural genes themselves. Once the chromosome assignment of the human globin genes was known, a metabolic marker closely linked to the globin structural genes would be chosen, and an erythroleukemia cell would be isolated which was deficient in that function. Fusion of this mutant MEL cell with human erythroid cells, and selection in a system which required the presence of the metabolic function, would result in a hybrid population dominated by hybrid cells containing the chromosome on which the human globin gene resides. Several human X mouse somatic cell hybrids containing a variable number of human chromosomes were used during the past year for the successful chromosomal localization of the human alpha and beta globin genes. Each clone was characterized as to the content of human chromosomes and as to the presence or absence of the human globin genes. Comparison of the chromosomal composition of the hybrid clones

with the presence or absence of the human alpha globin gene permitted identification of human chromosome 16 as the one which contains the human alpha globin locus. In addition, the availability of selective systems which could be used to isolate from the same hybrid clone cells which had retained or lost human chromosome 16 allowed us to establish that a complete correlation exists between the presence of human chromosome 16 and the human alpha globin gene.

3. Chromosomal Localization of the Human Beta Globin Structural Gene: The same and additional hybrid clones were used to study the chromosomal localization of the human beta globin gene. This analysis showed a correlation between human chromosome 11 and the human beta globin structural gene. While this work is still in progress, utilization of several human X mouse hybrid cells, each of which contained a different percentage of cells positive for human chromosome 11, permitted us to determine that a quantitative relationship existed between the percentage of cells which were positive for human chromosome 11 and the extent of the hybridization reaction used to detect the human beta globin gene.

4. Isolation of Mutant Mouse Erythroleukemia Cells Deficient in Markers Syntenic with the Human Globin Structural Gene: The presence of the enzyme adenine phosphoribosyl transferase on human chromosome 16 permitted us to select for retention or loss of this chromosome in hybrid cells as described above, and to establish that chromosome 16 contained the human alpha globin structural gene. Diaminopurine and 2-fluoradenine are compounds which are toxic to all cells containing the enzyme APRT, and were used for the isolation of clones of MEL cells resistant to these drugs. These latter cells were found to contain less than 0.05% of the APRT enzymatic activity present in normal cells. We will utilize these cells to isolate hybrids in which permanent retention of the human alpha globin structural gene occurs. These hybrid cells will then be used to study the state of the human globin gene sequences present in DNA during the induction of its transcription, and also to test whether there are regulatory elements governing globin gene expression which are located on human chromosomes other than 16.

5. Enrichment for the Globin Coding Region in a Chromatin Fraction from Reticulocytes by Endonuclease Digestion: One method of testing the state of the DNA during the induction of transcription is to test its sensitivity to nuclease digestion. Work over the past year (performed in the Experimental Hematology Section and the Institut für Molekularbiologie and Biochemie, Freie Universitat, Berlin-Dahlem, Germany) has led to the development of a method which permits one to use nuclease digestion of unique gene sequences to probe the state of the globin genes of erythroid and non-erythroid tissue (i.e., tissues in which expression of globin genes does and does not occur). Conditions were identified under which nuclease (DNase II) treatment results in the release of globin genes from DNA isolated from erythroid but not from non-erythroid cells. Currently, we are testing whether this sensitivity of the globin coding regions in the DNA of the MEL cell changes during

induction of the transcription of these genes. Such experiments may also be conducted with the human globin genes once they are introduced into MEL cells as described above. The release of the globin coding region from the DNA of such hybrid cells by DNase II treatment may permit the identification of proteins bound to this region. The characterization of such proteins and the definition of their role in the regulation of those structural genes which serve as markers for differentiated hematopoietic cells will be actively pursued.

6. Analysis of Expression of Markers of Different Hematopoietic Epigenotypes within a Single Hybrid Cell: Fusion of differentiated and undifferentiated cells (e.g., fibroblasts and hepatocytes) usually results in the loss of expression of the markers of the differentiated cell. In order to determine if the genetic rules governing commitment to the different lines of hematopoietic differentiation are identical or different from those governing commitment to the different differentiated tissue types in the body, hybrid cells derived from fusion of myeloid, erythroid, and lymphoid cells will be isolated. The pattern of the expression of the markers for erythroid, myeloid, and lymphoid differentiation will be tested in each hybrid cell to ascertain if coexpression of these markers or their extinction results from their introduction into a single cell. Stimulators of division of committed erythroid, myeloid, or lymphoid differentiated cells will also be used to test the sensitivity of hybrid cells derived by fusion of erythroid, myeloid cells or lymphoid cells to these cell-type-specific agents. In order to prepare for these studies, established erythroleukemia, myelogenous leukemia, and lymphatic leukemia cell lines were characterized with respect to the expression of the known markers for each of these lines of hematopoietic differentiation. The Jones Chloroma (rat myelogenous leukemia) cell line and the Friend Mouse Erythroleukemia cell line were tested by histochemical stains and by specific assays for the presence of markers of erythroid and myeloid differentiation. The Jones Chloroma exhibited expression of only myeloid phenotypes, while the MEL cell exhibited unique expression of erythroid phenotypes histochemically. The Jones Chloroma cells contained levels of alkaline phosphatase which were two logs lower than that present in the MEL cell by enzymatic assay. Jones Chloroma cells resistant to the purine analogue 6-thioguanine (and therefore deficient in the enzyme HGPRT) were isolated by selection from mass populations of cells. Thymidine kinase-deficient MEL cells were obtained. The presence of complementary mutations in these cells is necessary for selection of hybrid cells following fusion. The human lymphoid T and B cell lines "Molt" and "PA3B" were characterized with respect to the expression of specific lymphoid phenotypes. Thymidine kinase-deficient mutants have been obtained by selection from mass populations. The conditions necessary for the successful isolation of hybrid cells by fusion of these various hematopoietic cell lines are being identified. Following isolation of hybrid cells, the pattern of expression

of myeloid, erythroid, and lymphoid phenotypes will be characterized, as well as the responsiveness of the hybrid cells to cell type-specific stimulators of cell division (erythropoietic, colony stimulating factor, and lymphoid-specific mitogens.

7. A family in which a genetic predisposition to leukemia occurs has been studied with respect to viral-cell interaction and surface antigens. Previous studies had shown that members of the family destined to develop AML have fibroblasts which could be distinguished from the other normal family members when a quantitative SV40 transformation assay was used. In view of experiments suggesting that SV40 transformation of normal fibroblasts results in the appearance of new HLA specificities on cells, we are studying several members of the family to determine if they differ with respect to the HLA specificities identifiable on their fibroblasts before and after SV40 transformation. Preliminary results indicate that fibroblasts of some members develop new HLA specificities after SV40 transformation, while others do not. Since these studies are being performed in a blinded fashion, we cannot yet ascertain whether these differences are in any way related to the predisposition to AML.

Significance to Biomedical Research and the Program of the Institute:

Our objective has been to clarify the genetic and molecular mechanisms which control the orderly division and maturation of hematopoietic cells. Virtually all leukemic states involve a breakdown either in the orderly maturation of each type of differentiated cell or the complete arrest of the ability of these cells to mature normally, resulting in the accumulation of immature committed precursor cells and the cessation of production of the normal myeloid, erythroid, or lymphoid cells necessary to the survival of the patient. Clarification of the genetic mechanisms underlying these control processes in normal hematopoietic differentiation, as well as identification of the defective locus of control in the transformed state, may contribute to our ability to understand and to effectively treat these disorders.

Proposed Course:

Following completion of work designed to clarify the chromosomal locations of the human globin structural genes, an effort will be made to introduce single human chromosomes into mutant recipient mouse erythroleukemia cells. These cells will then be used to identify the chromosomal location of a gene which coordinates the expression of the human alpha and beta globin genes. The state of DNA in the human globin-gene-coding region during induction of transcription will be assessed by testing sensitivity to DNase II digestion. An attempt will be made to combine affinity chromotography, nucleic acid hybridization, and the genetic tools of cell fusion and gene transfer in order to obtain preparations of pure globin genes to use in isolation and study of proteins which regulate globin gene expression. Such regulatory substances are of potential use in defining the mechanisms of control in the differentiation and maturation of hematopoietic cells in normal and transformed states.

Publications:

1. Alter, B.P., Goff, S.C., Hillman, D.G., Deisseroth, A.B. and Forget, B.G.: Production of mouse globin in heterokaryons of mouse erythroleukemia cells and human fibroblasts. Life Science, in press.

2. Anderson, W.F., Deisseroth, A.B., Nienhuis, A., Gopalakrishnan, T.V., Huang, A., and Krueger, L.: Cellular and molecular studies on globin expression. Third Decennial Review Conference, the Tissue Culture Association, 1977, in press.

3. Anderson, W.F., Deisseroth, A.B., Velez, R., Nienhuis, A.W., Ruddle, F.H., and Kucherlapati, R.S.: A New Technique for Mapping Hemoglobin Genes. Human Gene Mapping 3, Vol. XII, No. 7: 367-371, 1976.

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7. Deisseroth, A., Nienhuis, A., Turner, P., Velez, R., Anderson, F., Ruddle, F., Lawrence, J., Creagan, R. and Kucherlapati, R.,: Localization of the human alpha globin structural gene to chromosome 16 in somatic cell hybrids by molecular hybridization assay. Cell, in press.

8. Deisseroth, A., Velez, R., Burke, R., Minna, J., Anderson, W.F., and Nienhuis, A.: Extinction of globin gene expression in human fibroblast X mouse erythroleukemia somatic cell hybrids. Somatic Cell Genetics 2: 373-384, 1976.

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10. Hendrick, D., Tolstoshev , P., and Randlett, D.,: Enrichment for the globin coding region in a chromatin fraction from chick reticulocytes by endonuclease digestion. Gene, in press.

11. Nienhuis, A., Barker, J., Deisseroth, A.B., and Anderson, W.F.: Regulation of globin gene expression. In Wetherall, D.J. (Ed.): Disordered erythropoiesis in Childhood, Ciba Foundation Symposium, Association Scientific Publishers, Amsterdam, pp. 329-347, 1976.

ANNUAL REPORT OF THE RADIATION ONCOLOGY BRANCH

NATIONAL CANCER INSTITUTE

July 1, 1976 through September 30, 1977

The past year witnessed substantial progress in several areas which were described in preliminary form in last year's annual report. In the laboratory, automated methodology has been developed for performing blastogenic assays as well as T and B cell analysis using rosetting and immunofluorescence techniques. These techniques are now operational and provide a rapid, sensitive, and reproducible means of quantitating various measures of immune function in patients entered into clinical trials. The automated system includes computerized printouts of serial measurements during the course of disease and/or treatment and without question is a potentially powerful adjunct to the many therapeutic trials ongoing in the Clinical Oncology Program.

Another area where significant technological advance has been forthcoming relates to the radiotherapy treatment planning system using computerized tomography. A partially operational system is now available with multiuser functions. Automatic boundary detection of normal structures has been implemented, scanner tomochemistry techniques (atomic number determination) have been developed and are being tested under calibration conditions, scanner image display has been expanded to include paper printouts in various formats, radiotherapy plan optimization using non-linear mathematics has been initiated, scanner dose distribution has been characterized and test methods presented to the BRH, FDA, HEW, and implemented in AAPM protocols, interactive software has been developed for the PDP-11 computer that permits irregular areas to be evaluated for area, mean CT number, noise, etc., TP-11 radiotherapy software has been adapted to PDP operation, and therapy beams have been characterized and parameterized for use in TP-11 planning. In addition, a survey contract to define specifications for dynamic therapy has been completed.

Emphasis on clinical investigation has continued with particular stress on a combined modality approach to small cell carcinoma of the lung. The study has been a highlight by demonstrating that durable, disease-free remissions are achievable in nearly one-half of patients presenting with tumor clinically confined to the chest at diagnosis. This program has provided the first evidence that a substantial fraction of patients with this heretofore rapidly fatal form of cancer may be potentially curable using currently available treatment methods. Other programs involving Hodgkin's disease, non-Hodgkin's lymphomas, and chronic lymphocytic leukemia have remained active while the study on Ewing's sarcoma was transferred to the Pediatric Oncology Branch. Collaborative studies involving soft tissue sarcomas, ovarian and pancreatic carcinoma, and several pediatric tumors have accessioned a significant number of patients during the year and are described in detail in annual reports of the respective collaborating Branches.

Consultative and service activities remained an important area of activity with over 360 patients being seen in formal consultation during the year and an additional 225 telephone consultations being logged. Non-clinical radiation exposures of animals and biological meterials were performed for 179 individuals together with "ad hoc" advice being provided on many occasions.

Finally, considerable effort was expended in the development of plans and design of a new radiotherapy treatment facility which will undergo construction in the fall of 1977. This facility will provide the National Cancer Institute a sophistication of exceptional degree for the prosecution of intramural radiotherapy studies in the years to come.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 00650-22 RO

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Service Radiation Therapy

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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OTHER:	C. A. Bull, M.D.	Expert Consultant	ROB	NCI
	K. Jentzsch, M.D.	Visiting Scientist	ROB	NCI
	M. K. Glover, R.N.	Clinical Nurse	ROB	NCI
	M. R. Powell, R.T.	Medical Radiation Tech.	ROB	NCI
	J. T. Anderson, R.T.	Medical Radiation Tech.	ROB	NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH
Radiation Oncology Branch

SECTION
Radiation Therapy Section

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
4.0	1.6	2.4

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of the project is to provide expert radiotherapy consultation and radiation treatment for Clinical Center patients admitted to services other than the Radiation Oncology Branch of the National Cancer Institute.

Project Description:

Objectives: To provide consultation and radiation treatment for Clinical Center patients.

Methods Employed: Formal and informal consultation with referring physicians and application of radiation therapy where appropriate with x-rays and electrons in accordance with standard radiotherapy practice as well as modified programs where necessitated by adjuvant concomitant therapies.

Major Findings: Over 360 patients were seen in formal consultation and an additional 225 logged telephone consultations provided "ad hoc" advice on treatment or general information. Approximately 368 patients will be treated in this fiscal year with over half of these being service patients, the remainder being protocol patients of the Radiation Oncology Branch.

Significance to Biomedical Research and the Program of the Institute: Provides essential radiation treatment support for ongoing clinical research programs of the National Cancer Institute and other Institutes with programs in the Clinical Center.

Proposed Course: To continue

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 00684-22 RO

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Non-Clinical Service Irradiation

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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LAB/BRANCH
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SECTION
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TOTAL MANYEARS: .3	PROFESSIONAL: .1	OTHER: .2

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Facilities and services have been provided to 179 individuals for the irradiation of animals and biological materials required for research studies by investigators at the National Institutes of Health. This includes consultation as to methods and types of irradiation and the preparation of material.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: To provide facilities and services as required for the irradiation of animals and biological materials for research study by investigators at the National Institutes of Health. This includes consultation as to methods and types of irradiation and the preparation of material.

Methods Employed: A dual 250 Kvcp X-ray generator is provided, output routinely calibrated, and maintained in proper operating condition.

Major Findings: The attached list indicates the organizational use of the irradiation facilities. About 72% of the total exposures were made for investigators in the National Cancer Institute. 179 individuals made use of the irradiation facilities during the past year.

Significance to Biomedical Research and the Program of the Institute: Same as Major Findings.

Proposed Course: It is again recommended that consideration be given to the purchase of a self-contained cesium irradiator at a cost of about $60,000. 60% of the present users of the X-ray equipment have indicated a desire for this equipment. Since this equipment is not electronic in nature, more uniform dosimetry would result. Maintenance of this type of equipment is minimal and would reduce the maintenance problems of the X-ray equipment.

Publications: None except as the use of these facilities are indicated in publications by the various investigators.

Services Provided Using the Dual 250 KVCP X-ray Equipment from April 1, 1976 to April 1, 1977

Organization	No. of Exposures
A-A&R	10
A-CE	8
A-DSD	1
A-LCI	111
A-LMI	105
A-LPB	23
A-LVD	120
* NIAID	10
BB-DBBP	514
BB-DV	189
C-B	49
C-BCRC	5
C-CB	6
C-D	370
C-EXP	21
C-I	296
C-LB	36
C-LCB	92
C-LCBGY	57
C-LIB	51
C-LID	163
C-LMBGY	2
C-LMCB	33
C-LMPH	406
C-LPHY	1155
C-LRNATV	19
C-LTCB	1
C-LVC	87
C-M	95
C-PO	9
C-RO	17
C-SURG	45
C-VI	80
C-VLL	27
C-VO	24
* C-DCCP	68
* NCI	259
CH-BB	14
* NICHD	5
D-LDBA	12
D-LMI	165
I-LI	307

Organization	No. of Exposures
I-LMI	13
I-LPD	15
N-LEN	15
N-NI	75
** Microbiological	912
** Bionetics	96
** Meloy	3

* Individual branches were not specified.

** Exposures for Microbiological Associates, Litton Bionetics and Meloy Laboratories were made on materials for projects under contracts with the National Cancer Institute.

Materials irradiated included cells, tissue culture, blood parasites, mosquitoes, mice, rats, hamster, chickens, guinea pigs and monkeys. Exposures ranged from 25R to 30,000R.

It is estimated that as many as 20% of exposures made were not recorded.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 00988-12 RO

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

The Study of Chronic Lymphocytic Leukemia

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	R. E. Johnson, M.D.	Chief	ROB	NCI
OTHER:	Staff of the Rad. Oncol. Branch		ROB	NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH
Radiation Oncology Branch

SECTION
Office of the Chief

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.0	1.5	.5

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

It is the long range purpose of this project to study the natural history of chronic lymphocytic leukemia, to define those disease parameters which indicate the need for early therapeutic intervention, and to investigate new therapeutic approaches which will improve the quantity and quality of survival.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: The study is directed toward understanding the epidemiology, natural history, and management of chronic lymphocytic leukemia.

Methods Employed: One hundred patients with previously untreated chronic lymphocytic leukemia have been accessioned into this study. Information related to carcinogenic exposure, family history, the natural history of disease, immunologic parameters, treatment and response (with primary emphasis on total body irradiation), and survival is being collected and computerized for analysis.

Major Findings: It has been determined for the first time that treatment can alter the natural history of patients with active, progressive chronic lymphocytic leukemia and not only prolong survival but improve the quality of life for approximately one-third of patients.

Significance to Biomedical Research and the Program of the Institute: This study has provided the first documented evidence that treatment of patients with chronic lymphocytic leukemia can quantitatively and qualitatively alter the course of disease.

Proposed Course: Accession to the study has been terminated. The treatment and followup of patients currently onstudy will continue in the ROB for the present time. As this population of patients constitutes a valuable research resource, it would be anticipated that another Branch would absorb the study if a decision is made to terminate the ROB's primary responsibility by the incoming Chief.

Publications:

Johnson, R. E.: Total body irradiation of chronic lymphocytic leukemia: Relationship between therapeutic response and prognosis. Cancer 37: 2691-2696, 1976.

Johnson, R. E., and Ruhl, U.: Treatment of chronic lymphocytic leukemia with emphasis on total body irradiation. Intern. J. Rad. Oncol. 1: 387-398, 1976.

Brayland, R. C., Jaffe, E. S., Burbach, J. W., Frank, M. M., Johnson, R. E., and Berard, C. W.: Similarities of surface characteristics of neoplastic well-differentiated lymphocytes from solid tissues and peripheral blood. Cancer Res. 36: 1619-1627, 1976.

Johnson, R.E.: Role of radiation therapy in management of adult leukemia. Cancer 39: 852-855, 1977.

Johnson, R.E.: Radiotherapy as primary treatment for chronic lymphocytic leukemia. Clin. Haematal. 6: 237-244, 1977.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 00989-12 Ro

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

The Study of Hodgkin's Disease

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	R. E. Johnson, M.D.	Chief	ROB	NCI
OTHER:	Staff of the Rad. Oncol. Branch		ROB	NCI

COOPERATING UNITS (if any)
Medicine Branch, NCI
Laboratory of Pathology, NCI

LAB/BRANCH
Radiation Oncology Branch

SECTION
Office of the Chief

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS: 1.5	PROFESSIONAL: 1	OTHER: .5

CHECK APPROPRIATE BOX(ES)
☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

It is the long range purpose of this project to study the natural history of Hodgkin's disease, to assess the reliability of diagnostic methods as they apply to the treatment decisions, to examine the effectiveness of radiotherapy in the management of disease, and to evaluate the long-term survival and the iatrogenic effects of therapy.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: To understand the natural history of Hodgkin's disease, assess the reliability of diagnostic methods as they apply to the treatment decisions, to examine the effectiveness of radiotherapy in the management of disease, and to evaluate the long-term survival and the iatrogenic effects of therapy.

Methods Employed: A collaborative effort involving a number of cooperating units was undertaken to document the biological characteristics of each patient's disease including the extent of involvement. Subsequently, patients were assigned to various treatment options to test the comparative therapeutic efficacy.

Major Findings: During the past year, there has been no significant change in previously reported survival rates. Careful assessment has been undertaken of the long-term effects of treatment with particular emphasis on the hematologic and immunologic aspects.

Significance to Biomedical Research and the Program of the Institute: This study has demonstrated the merit of extensive prophylactic irradiation in the management of clinical early stage Hodgkin's disease. Information has also been forthcoming as to the relative accuracy of various diagnostic tests and the definition of which situations require unusual pretreatment procedures such as staging laparotomy.

Proposed Course: Continued long-term observation of patients without accession of additional patients to study.

Publications:

Johnson, R.E., Glover, M., Johnson, S.K., and Ruhl, U.: Split-course radiotherapy of Hodgkin's disease: local tumor control and normal tissue reactions. Cancer 37: 1713-1717, 1976.

Kun, L.E., DeVita, V.T., Young, R.C., and Johnson, R.E.: Treatment of Hodgkin's disease using intensive chemotherapy followed by irradiation. Intern.J.Rad.Oncol. 1: 619-626, 1976.

Johnson, R.E., Zimbler, H., Berard, C.W., Herdt, J., and Brereton, H.D.: Radiotherapy results for nodular sclerosing Hodgkin's disease after clinical staging. Cancer 39: 1439-1444, 1977.

Johnson, R.E.: The Role of Selective Staging Laparotomy in Hodgkin's Disease and the Malignant Lymphomas. In Varco and Delaney (Eds.): Controversy in Surgery. Philadelphia, W.B. Saunders, 1976, pp. 349-358.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 00990-12 Ro

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

The Study of Non-Hodgkin's Lymphomas

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	R. E. Johnson, M.D.	Chief	ROB	NCI
OTHER:	H. D. Brereton, M.D.	Head, Rad. Med. Sec.	ROB	NCI
	Staff of the Rad. Oncol. Branch		ROB	NCI

COOPERATING UNITS (if any)
Medicine Branch, NCI
Laboratory of Pathology, NCI

LAB/BRANCH
Radiation Oncology Branch

SECTION
Office of the Chief

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS: 2	PROFESSIONAL: 1.5	OTHER: .5

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TI UES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this study is to increase our understanding of the natural history of the various malignant lymphomas, to define which diagnostic (staging) methods are requisite for making treatment decisions, and to evaluate various therapeutic approaches which might improve the survival and quality of life for patients with these diseases.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: To improve our understanding of the natural history of malignant lymphomas, to define which staging methods are required for effective treatment decision-making, and to evaluate new therapeutic approaches with the aim of improving both survival and quality of life.

Methods Employed: Extensive prospective staging has been completed on well over two hundred patients, which patients have subsequently been accessioned into clinical trials to examine the comparative value of different therapeutic approaches.

Major Findings: The systemic nature of involvement in the overwhelming majority of patients with non-Hodgkin's lymphoma has been established by this collaborative study with the Medicine Branch and Laboratory of Pathology. This information has served to alter the concepts regarding treatment and the more routine use of systemic therapy has resulted. However, while remissions can be achieved in the majority of patients using either radiotherapy or chemotherapy, relapse eventually occurs in most patients and the investigation of alternative therapeutic measures will be required.

Significance to Biomedical Research and the Program of the Institute: This study has contributed to our understanding of non-Hodgkin's lymphoma primarily in terms of understanding the natural history and providing information as to methods of staging patients (i.e., identifying their location in the overall spectrum of disease). The remaining challenge is to develop more effective means of therapy in the future based on the above understanding.

Proposed Course: Continued accession of patients is anticipated for the purpose of examining new treatment approaches.

Publications:

Chabner, B.A., Johnson, R.E., Young, R.C., Canellos, G., Hubbard, S., Johnson, S.K., and DeVita, V.T.: Sequential non-surgical and surgical staging of non-Hodgkin's lymphoma. Ann.Int.Med. 85: 149-154, 1976.

Young, R.C., Johnson, R.E., Canellos, G.P., Chabner, B.A., Brereton, H.D., Berard, C.W., and DeVita, V.T.: Advanced lymphocytic lymphoma: randomized comparisons of chemotherapy and radiotherapy, alone or in combination(In Press).

Reimer, R.R., Chabner, B.A., Young. R.C., Reddick, R., and Johnson, R.E.: Lymphoma presenting in bone: results of staging, histopathology, and therapy. Ann.Int.Med.(In Press).

Brereton, H.D., Fairey, R.N., and Johnson, R.E.: Successful chemotherapy of advanced non-Hodgkin's lymphoma after relapse following total body irradiation. Intern.J.Rad.Oncol.(In Press).

Johnson, R.E.: The Role of Selective Staging Laparotomy in Hodgkin's Disease and the Malignant Lymphomas. In Varco and Delaney (Eds.): Controversy in Surgery. Philadelphia, W.B. Saunders, 1976, pp. 349-358.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF
HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 CM 00994-06 RO

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

The Study of Small Cell Cancer of the Lung

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	R. E. Johnson, M.D.	Chief	ROB	NCI
OTHER:	H. D. Brereton, M.D.	Head, Rad. Med. Sec.	ROB	NCI
	C. H. Kent, M.D.	Head, Rad. Ther. Sec.	ROB	NCI

COOPERATING UNITS (if any)

Diagnostic Radiology Dept., CC, NIH
Nuclear Medicine Dept., CC, NIH

LAB/BRANCH
Radiation Oncology Branch

SECTION
Office of the Chief

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS: 2.5	PROFESSIONAL: 2.0	OTHER: .5

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This study is concerned with the evaluation of methods for pretreatment assessment of patients, identification of factors influencing response to therapy, and investigation of combined modality treatment using radiotherapy and intensive chemotherapy.

Project Description:

Objectives: The major objective of the program is to improve the survival time for patients with small cell carcinoma of the lung and hopefully to develop treatment programs which may offer some prospect of cure.

Methods Employed: Seventy patients with previously untreated disease have been treated since November 1974. A diagnostic workup consisting of physical examination, whole lung tomography, bone marrow biopsy, and radioisotope scans of bone, liver, and brain has been employed to divide the cases into two categories, i.e., those with disease limited to the chest (intrathoracic group) and those with extrathoracic metastases. The therapeutic approach utilizes combination chemotherapy, whole brain irradiation, and radiotherapy to regional disease.

Major Findings: The complete remission rate for intra- and extrathoracic disease has been approximately 80% and 50% respectively. Following the induction course of treatment, lasting 3-4 months, patients have been observed without further treatment when a complete remission was noted. The duration of remission for patients with extrathoracic disease has been relatively short without maintenance therapy (median 5.6 months) but survival has been significantly prolonged (median 10.5 months) as compared to historical controls. In contrast, durable unmaintained remissions have been observed in one-half of patients with intrathoracic disease who achieved complete remissions and the median survival has been dramatically prolonged (median in excess of 2 years).

Significance to Biomedical Research and the Program of the Institute: The durable remissions in a significant fraction of patients with more limited disease suggests the possibility of cure in this heretofore fatal form of lung cancer and constitutes a major therapeutic advance.

Proposed Course: Accession of patients is continuing with the goal of improving the disease-free rate and developing techniques for combining chemotherapy and radiotherapy without incurring serious toxicity as experienced by a significant fraction of patients treated during the early phase of the study.

Publications:

Johnson, R.E., Brereton, H.D., and Kent, C.H.: Small cell carcinoma of the lung: attempt to remedy cause of past therapeutic failure. Lancet 2: 289-291, 1976.

Greco, F.A., Brereton, H.D., Kent, C.H., Zimbler, H., Merrill, J., and Johnson, R.E.: Adriamycin and enhanced radiation reaction in normal esophagus and skin. Ann.Int.Med. 85: 294-298, 1977.

Kent, C.H., Brereton, H.D., and Johnson, R.E.: "Total" therapy for oat cell carcinoma of the lung. Int.J.Rad.Oncol.(In Press).

Brereton, H.D., Kent, C.H., and Johnson, R.E.: Chemotherapy and radiation therapy for small cell lung cancer: a remedy for past therapeutic failure. Proceedings of the Second Conference on Lung Cancer Treatment(In Press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 00995-02 RO

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Radiation Therapy Planning Using Computerized Tomography Interfaced with Computerized Dosimetry Systems

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	L. R. Kirkland, Ph.D.	Physicist, Head	ROB	NCI
OTHER:	R. E. Johnson, M.D.	Chief	ROB	NCI
	D. Syed, M.S.E.E.	Head, CSL	CR	NIH
	W. L. Risso, M.E.	Elec. Engineer	CR	NIH
	J. Knight, B.S.	Computer Systems Analyst	CR	NIH
	H. A. Fredrickson	Computer Systems Analyst	CR	NIH
	J. L. Christensen, M.S.	Elec. Engineer	CR	NIH
	H. Lee, Ph.D.	Visiting Scientist	CR	NIH

COOPERATING UNITS (if any)

Computer Systems Laboratory, CR. NIH

LAB/BRANCH
Radiation Oncology Branch

SECTION
Radiation Physics and Computer Automation Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS: 4.5	PROFESSIONAL: 4.5	OTHER:

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project is directed toward utilizing computerized tomography to improve radiation therapy. An EMI whole-body scanner has been installed and upgraded to a production model CT5005. Ancillary computer equipment for the computerized radiation therapy treatment planning system has been installed. Software is being modified to permit the use of the advanced imaging resolution and inhomogeneity density identification for improving treatment plans. Such software includes automatic boundary detection, irregular area mean density with standard deviation and paper copies of images in various formats, resolutions, and gray scales.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: To utilize the imaging from computerized tomography for improved accuracy in radiation treatment planning and to employ data on tissue densities inherent in the scanning process to correct for tissue inhomogeneities in dosimetry planning.

Methods Employed: A closely integrated effort is being undertaken by the staff of the Radiation Oncology Branch and the Computer Systems Laboratory, Division of Computer Research and Technology, to implement the study. The whole body scanner has been installed, with final acceptance testing currently ongoing. The ancillary computer equipment has been installed and programmed to accommodate radiotherapy treatment planning software. This software is being modified to allow for existing therapy equipment and required display options. Special software has been written for automatic boundary detection within patient images obtained on magnetic tape from the scanner. The software will yield intrascan area and density for contours as well as interscan volume and density for organs, tumors, etc., that can be imaged. Paper output of images scaled to life size have been obtained and used for treatment planning with an existing computer.

Major Findings: Corporate reluctance to permit data transfer between scanner and the treatment planning system can be circumvented by carefully adjusting the independent tape drive of each system and transferring magnetic tape reels. Existing reconstruction area of scanner must be expanded to at least 16 inches in diameter in order to eliminate edge cut-off in large patient images. Computer-physician interaction time can be reduced by almost a factor of 10 for contour entry by using automatic boundary detection based upon stored density information. Images can be displayed on paper in various formats and resolutions and scaled to life size with spatial errors of less than 1%. Images can be displayed on advanced video monitors with 16X video magnification to aid image analysis.

Significance to Biomedical Research and the Program of the Institute: A successful outcome to this program will improve the accuracy of treatment planning, hence dose delivery, to patients undergoing therapy on research protocols as well as significantly improving the sophistication of radiation therapy throughout the world.

Proposed Course: Expedient prosecution

Publications:

Kirkland, L.R., and Morgan, T.J.: Commissioning of computed-tomography scanners-an overview. Proceedings of the Society of Photo-Optical Instrumentation Engineers 96: 220-227, 1976.

Kirkland, L.R., Syed, D., Risso, W.L., Morgan, T.J., and Smith, L.B.: The role of CT scanners in radiotherapy. Proceedings of the Society of Photo-Optical Instrumentation Engineers 96: 269-273, 1976.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 00996-01 RO

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Immunologic Function in Patients Receiving Total Body Irradiation

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: J. D. Lamberg, M.D. Senior Investigator ROB NCI

COOPERATING UNITS (if any)

LAB/BRANCH
Radiation Oncology Branch

SECTION
Radiation Medicine Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS: 1.75	PROFESSIONAL: .75	OTHER: 1.0

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Total Body Irradiation has been shown to cause striking regression of disease in chronic lymphocytic leukemia (CLL) and in non-Hodgkin's lymphoma (NHL). The time course of the response, particularly in NHL, suggests that mechanisms other than radiation-induced cell death may be involved, and further that the mechanisms may be immunologic. The time course of response, 6-8 weeks in NHL and 6-12 months in CLL, are such that these diseases also become suitable models in which to develop and test serial immunologic monitoring techniques. This study was initiated in order to: (1) develop tests of immunologic function which are sufficiently reproducible, and sensitive to detect alterations in immune function induced by therapy and/or change in disease status; and (2) apply these tests in a situation where it is likely that one may be able to assess the relative contributions of therapy and level of immune function to change in disease status.

Project Description:

Objectives: Although many assays of immunologic function have been developed and applied in the last decade, inattention to many relevant biological and technical factors has made the interpretation of their significance and relevance almost impossible. This situation is further complicated by the tendency of many studies to assume at the outset that immunologic alterations are the relevant alteration in the outcome of the disease process, and then to present data on immunologic alterations as the proof of this pre-drawn conclusion of relevance.

Although a body of knowledge has been developed which suggests that immune function and the development of cancer are related, the exact nature of this relationship, and ways in which it may be exploited to clinical advantage are largely unknown. If one is to approach the question scientifically, one first needs to develop reliable, reproducible tests which can serve as true yardsticks for hypothesis testing.

Total Body Irradiation has been shown to cause striking regression of disease in chronic lymphocytic leukemia (CLL) and in non-Hodgkin's lymphomas (NHL). The time course of response, particularly in NHL, suggests that mechanisms other than radiation induced cell death may be involved and further that the mechanisms may be immunologic. Studies have shown that Total Body Irradiation causes immunological depression and recovery in rats, (Lamberg, 1971) and its time course is similar to that of response in NHL. The time course of response, 6-8 weeks in NHL and 6-12 months in CLL are such that these diseases also become suitable models in which to develop and test serial immunologic monitoring techniques.

This study was initiated in order to (1) develop tests of immunologic function which are sufficiently reproducible and sensitive to detect alterations in immune function induced by therapy and/or change in disease status and (2) apply these tests in a situation where it is likely that one may be able to assess the relative contributions of therapy and level of immune function to change in disease status.

Methods Employed:

(1) Skin testing
(2) *In vitro* blastogenesis
(3) T & B cell analysis using rosetting and immunofluorescence techniques

Major Findings: Initial studies indicate that (1) serum factors, which have usually been held unimportant, have a significant impact on the *in vitro* reactivity to blastogenic assays as measured with tritiated thymidine uptake as an endpoint; (2) tritiated thymidine uptake is a poor endpoint for blastogenic assays; (3) automated methods of counting cell number may be used as an endpoint for blastogenic assays, and also for quantitating rosette formation.

Significance to Biomedical Research and the Program of the Institute: A considerable developmental research effort has been based on the assumption of the relevance of immune function to carcinogenesis and cancer therapy. Nevertheless, the initial fruits of such research, as presented at the recent Immunotherapy Symposium (Terry, 1976) were indeed disappointing. It is time for critical thinking and for the development of objective and sensitive yardsticks for hypothesis testing. The particle counting system developed in our laboratory is likely to provide tests meeting this need. To proceed rationally we need accurate and reproducible tests by which to analyse immune function. We may then be in a position to analyse mechanisms of failure in immunotherapy trials, and also to do the same in trials employing immune modulation.

Proposed Course: The methods which have been recently developed will be employed in normal subjects and patients and then subjected to technical analysis when approximately 10 patients and normals have been studied. Thereafter, tests will be performed until statistically significant correlations between treatment, immune status and disease response have been established or refuted.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 00997-01 RO

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Service Radiation Physics

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	L. R. Kirkland, Ph.D.	Head, Rad. Physics Sec.	ROB	NCI
OTHER:	R. W. Swain, M.S.	Physicist	ROB	NCI
	A. Wheatley, B.S.	Elec. Technician	ROB	NCI
	R. Whittaker	Eng. Technician	ROB	NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH
Radiation Oncology Branch

SECTION
Radiation Physics and Computer Automation Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.5	1.5	2.0

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose is to provide expert radiation physics consultation and services to the Radiation Oncology Branch for Branch patients as well as Clinical Center patients admitted to other services.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: To provide expert physics consultation and services to physicians who have patients undergoing radiation therapy. Areas of consultation include radiation therapy treatment planning, equipment selection, computers, computer programs, radiation dosimetry, and radiation safety. Areas of service include maintenance and calibration of radiation-producing equipment, design and fabrication of devices that aid in the delivery of medical services to Branch patients, patient data survival analysis, and protective barrier review.

Methods Employed: Formal and informal consultations with physicians and staff when appropriate. Routine services are accomplished by using tools and techniques as required for each service.

Major Findings: Services and consultations were provided for over 360 patients treated by Branch physicians. X-ray sources in the diagnostic energy range were mounted onto high-energy linear accelerators to provide better patient portal films. Computer programs were written to expedite the analysis of patient files stored on Institute computers. Protective barriers for the planned therapy building were reviewed. Subsequent recommendations concerning appropriate shielding resulted in savings of approximately $500,000.00 in construction costs.

Significance to Biomedical Research and the Program of the Institute: Provides essential radiation physics support for ongoing clinical research programs of NCI and other Institutes with programs in the Clinical Center.

Proposed Course: To continue

Publications: None

ANNUAL REPORT SUMMARY

SURGERY BRANCH

NATIONAL CANCER INSTITUTE

July 1, 1976 to September 30, 1977

Clinical efforts in the Surgery Branch continue to emphasize combined modality approaches to the treatment of cancer. Active clinical protocols are in progress for the treatment of osteosarcoma, soft tissue sarcomas, melanoma, rectal cancer, testicular cancer, and lung cancer. Immunotherapeutic approaches to cancer treatment are being explored. Active laboratory research programs in tumor immunology and tumor metabolism are in progress.

Surgical procedures and consultations performed by the Surgery Branch from April 1, 1976 to March 31, 1977 are presented in Tables I to IV.

TABLE I
Cases - General Surgery*
April 1, 1976 - March 31, 1977

General:	Exploratory laparotomy	39
	Splenectomy	15
	Colon/bowel resection	12
	Abdominal-perineal resection	4
	Gastrectomy	10
	Pancreatectomy - partial	3
	Biliary by-pass	9
	Cholecystectomy	9
	Major soft tissue or muscle group excision	17
	Revision of colostomy	2
	Creation loop colostomy	5
	Herniorrhaphy/hernia repair	7
	Wound exploration	5
	Electrofulguration - rectum	14
	Colonoscopy	19
	Laparoscopy	8
	Peritoneoscopy	5
Surgery for Melanoma:	Wide excision	27
	Wide excision + Axillary LND	19
	+ Inguinal ± deep LND	16
	+ Radical neck LND	9
	Lymph node dissection: Axillary	11
	Inguinal ± deep	21
	Radical neck	4
Head & Neck:	Radical neck ± other resection	11
	Other major cases	2
	Tracheostomy	8
	Bronchoscopy	2
	Direct laryngoscopy/pharyngoscopy	4
Plastic:	Removal Schribner shunt	4
	Construction/revision Schribner shunt	9
	Wound debridement - major	11
	Construction/revision/movement - flaps	19
	STSG	7
	Electrofulguration skin lesions	3
G-U:	Retroperitoneal node dissection	5
	Debulking procedure	4
	Orchiectomy/orchiopexy	7

TABLE I (continued)

G-U: (continued)	Nephrostomy/nephrolithotomy/nephrectomy	4
	Suprapublic prostatectomy	2
	TURP	10
	Testicular biopsy, blood sampling	8
	Repair varicocele	2
	Loop revision	4
	Suprapubic cystostomy	4
	Vasectomy	1
	Circumcision	1
	Cystoscopy with or without other studies, or biopsy	70
	Looposcopy	5
Breast:	Breast biopsy	57
	Simple mastectomy	4
	Modified radical mastectomy	12
	Reduction mammoplasty	7
GYN:	Hysterectomy	2
	Oophorectomy	3
	D and C	3
Amputations:	Below knee	1
	Above knee	8
	Hip disarticulation	6
	Hemipelvectomy	5
	Shoulder disarticulation	1
	Forequarter amputation	3
	Stump revision	1
Endocrine:	Parathyroidectomy	19
	Parathyroid implant or removal	5
	Adrenalectomy	8
	Thyroidectomy - complete or subtotal	19
Minor:	I and D	7
	Biopsy: Temporal artery	2
	Bone	14
	Node	107
	Tissue mass	107
Miscellaneous:		7

*In compiling this list the major aspect of each procedure was used. Patients with multiple simultaneous procedures appear on the list only one time.

TABLE II
Consultants - Surgery
April 1, 1976 - March 31, 1977

Plastic:	
Mammoplasty	6
Grafting, extensive	2
Dermabrasion	1
Excision scar	3
Thoracic:	
Pericardectomy	1
Bronchoscopy	10
Thoracotomy - nodules	48
Thoracotomy - infiltrates	16
Thoracotomy - other	1
Lobectomy	10
Pneumonectomy	2
Mediastinoscopy	9
Rib resection	2
Miscellaneous	6
GYN:	
Hysterectomy	1
D&C	1
Salpingectomy	1
ENT:	7
Vascular:	2

TABLE III

Individual Written Intramural Consultations Received by Surgery Branch, NCI Listed by Institutes*

April 1, 1976 - March 31, 1977

NCI	100
NIAMDD	102
NHLBI	38
NIAID	80
NIMH	39
NINCDS	47
NIDR	8
NICHHD	8
NEI	11
TOTAL	433

*This tabulation includes only formal written consultations submitted to the Surgery Branch office. Many additional consultations are received by Surgery Branch physicians directly by telephone or personal contact. These are not included in this tabulation.

TABLE IV
Annual Statistical Report
Surgical Nursing Service
April 1, 1976 - March 31, 1977

Patient		NCI	NHLBI	NINCDS		NIAID	NIAMDD	NEI	NIMH	NICHHD	NIDR	Consult-ants	Total #Pts. Operated
				Neuro.	Muscle								
NCI	Major	551	47	3	0	0	1	1	0	0	1	19	623
	Minor	295	4	2	1	0	1	3	0	0	0	6	312
NHLBI	Major	32	270	0	2	0	0	0	0	0	1	0	305
	Minor	17	12	0	1	0	0	1	0	0	0	1	32
NINCDS	Major	10	15	38	7	0	0	0	0	0	0	1	71
	Minor	4	2	3	256	0	0	1	0	0	0	2	268
NIAMDD	Major	58	0	3	0	0	13	0	0	0	1	4	79
	Minor	22	0	0	6	0	6	0	0	0	0	0	34
NIAID	Major	29	2	1	0	0	0	3	0	0	0	4	39
	Minor	15	0	0	6	0	0	1	0	0	0	3	25
NIDR	Major	0	0	0	0	0	0	0	0	0	1	0	1
	Minor	0	0	0	1	0	0	0	0	0	0	0	1
NIMH	Major	1	2	2	0	0	0	0	0	0	0	0	5
	Minor	4	0	0	0	0	0	0	0	0	0	0	4
NICHHD	Major	20	2	2	1	0	0	1	0	0	0	2	28
	Minor	2	0	0	2	0	0	0	0	0	0	0	4
NEI	Major	1	0	0	0	0	0	34	0	0	0	0	35
	Minor	1	0	0	1	0	0	11	0	0	0	0	13
MAJOR TOTAL		702	338	49	10	0	14	39	0	0	4	30	1186 +1(Anes.)
MINOR TOTAL		360	18	5	274	0	7	17	0	0	0	12	693 +1(Anes.)
GRAND TOTAL		1062	356	54	284	0	21	56	0	0	4	42	1879 +2(Anes.) *1881

*Of these 1881 surgical procedures, 160 patients were on OPD status.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 03800-07 SURG

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Surgical Consultations and Collaborative Research Involving Surgical Services at the National Institutes of Health

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	S. A. Rosenberg	Chief of Surgery, NCI	SURG	NCI
	and entire staff of	Surgery Branch, NCI	SURG	NCI
Other:	G. D. Aurbach	Chief, Metabolic Dis. Br.	MD	NIAMDD
	F. C. Bartter	Clinical Director	IR OD	NHLBI
	J. L. Doppman	Chief, Diag. Radiol. Dept.	DR	CC
	R. E. Johnson	Chief, Rad. Oncol. Br.	RO	NCI
	J. Robbins	Chief, Clin. Endocrin. Br.	CE	NIAMDD
	L. B. Thomas	Chief, Lab. of Pathology	LP	NCI
	R. C. Young	Chief, Medicine Branch	M	NCI
	A. S. Levine	Chief, Ped. Oncol. Br.	PO	NCI

COOPERATING UNITS (if any)

LAB/BRANCH
Surgery Branch

SECTION
Office of the Chief

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
6.0	4.0	2.0

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The Surgery Branch of the National Cancer Institute are the general surgeons and general surgical consultants to the entire National Institutes of Health. In this role we see patients for elective consultations as well as all emergency general surgical problems. Many collaborations on clinical studies have resulted from these consultative efforts.

Introduction

Investigators in the Surgery Branch of the National Cancer Institute are the general surgeons and general surgical consultants to the entire National Institutes of Health. In this role we see patients in primarily two capacities. Firstly, we see patients in consultation for all general surgical and specialty surgical problems except for the specialties of cardiac and orthopedic surgery. The Surgery Branch answers all emergency as well as elective surgical consultations and provides 24 hour coverage for surgical emergencies that may arise in the Clinical Center Hospital.

Secondly, the Surgery Branch collaborates in the procurement of tissues for studies required by other investigative units. The degree of involvement of the Surgery Branch in the planning and execution of these studies is variable. The Surgery Branch often plays an instrumental role in the design of these studies while in other collaborations, the Surgical Service merely provides tissues.

Approximately 40% of the clinical surgical effort of the Surgery Branch is devoted to these consultative and collaborative studies.

A complete listing of our surgical procedures is presented in Table I. Surgery performed by surgical consultants operating within the Surgery Branch is listed in Table II.

A list of consultations received from other NCI Branches as well as other NIH Institutes is shown in Table III.

Project Description:

Part I.	Hyperparathyroidism
Part II.	Thyroid Studies
Part III.	Reduction Mammoplasty
Part IV.	Evaluation of the Countercurrent Exchange of Testosterone in the Spermatic Artery and Vein of Men with Infertility
Part V.	Nutritional Support
Part VI.	Ovarian Cancer

Project Description:

Part I. Hyperparathyroidism

Objectives:

The Surgery Branch of the National Cancer Institute is actively involved in a major clinical commitment with the Section of Mineral Metabolism of the

Metabolic Diseases Branch of the National Institute of Arthritis, Metabolism, and Digestive Diseases along with the Diagnostic Radiology Branch of the Clinical Center. This is an ongoing study of the diagnosis, localization, and treatment of hyperparathyroidism. The Surgery Branch provides operative support of an extensive clinical investigative protocol.

Methods Employed:

The majority of cases referred have had multiple, previous unsuccessful surgical attempts to relieve their disease. Standard investigative protocols are followed. Emphasis on thyroid arteriography and venous sampling for localization continues. Computerized axial tomography has been employed as a screening test.

It should be emphasized that the collaborative effort has been expanded by our efforts at autotransplantation of patients who have had their solitary gland removed. Of particular interest is our ability now to cryopreserve parathyroid tissue and await the development of the aparathyroid or hypoparathyroid state and then transplant the patients. Preliminary experience with this technique has given us some rather exciting success. In those patients who have functioning parathyroid autografts in the forearm we now have an *in vivo* model for the study of parathyroid function.

Major Findings:

In the period 8/20/75 to 3/15/77 thirty-seven patients with hyperparathyroidism have been operated on. Of these, 25 have had a total of 53 previous procedures. The outcome of 24 of the reoperative cases is illustrated in Table V.

Significance to Cancer Research and the Program of the Institute:

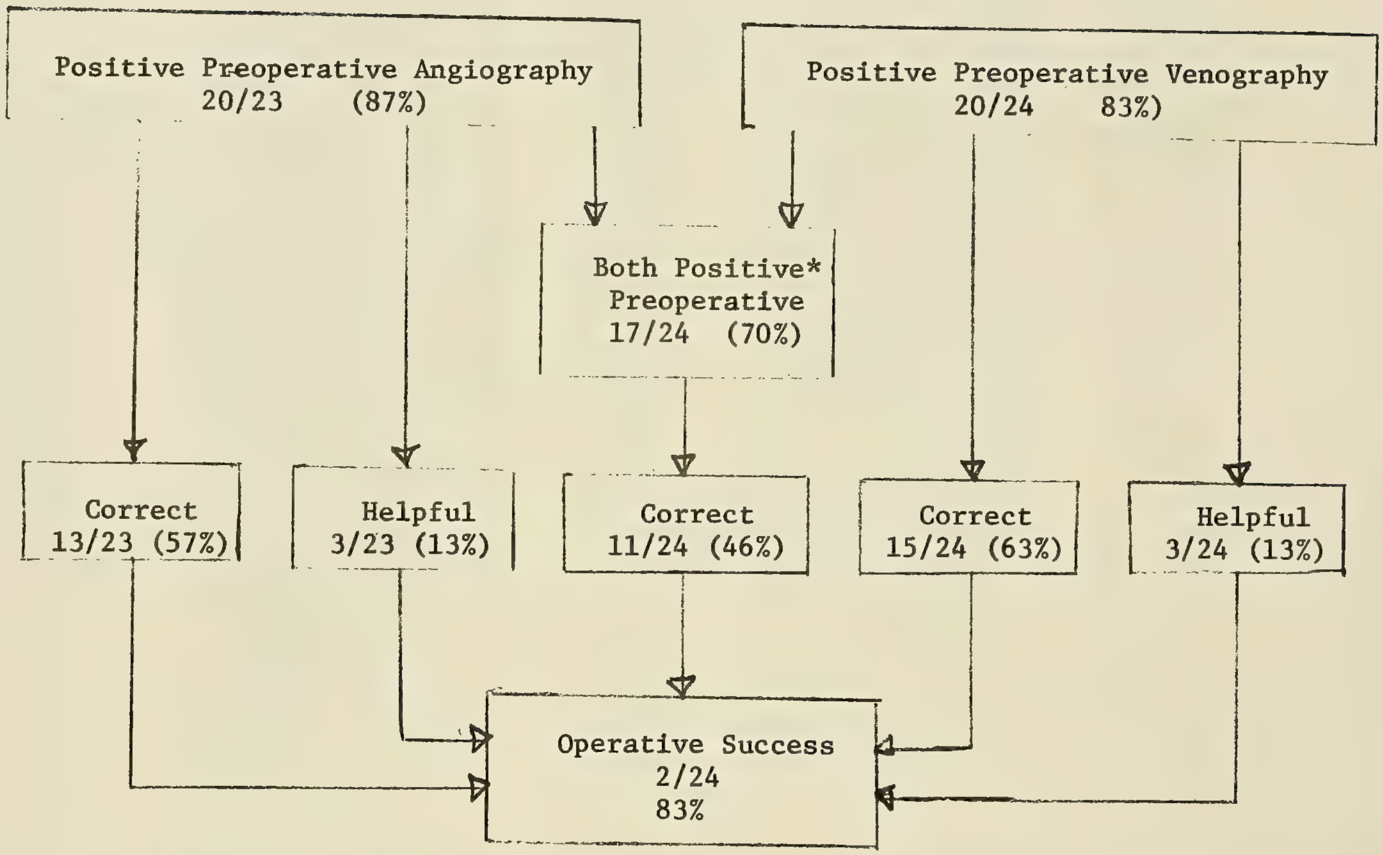
The surgery of this disease is an essential end point in the therapy and management of patients with hyperparathyroidism. By early diagnosis and successful surgery an important step can be taken towards the prevention of the complications of prolonged hypercalcemia. These primarily manifest in our experience with end-stage renal disease. The Institute provides a national service in providing a referral center for those patients previously operated on unsuccessfully elsewhere. By the use of the extensive preoperative evaluation and localization techniques, successful reoperation should be obtainable.

Proposed Course:

The project continues and is expected to increase as the national reputation of the medical and radiological groups involved extends. The Surgery Branch will be actively involved in further development of the cryopreservation techniques, to determine the need for autotransplantation and in the prolonged long-term evaluation of the course of patients and their transplanted parathyroid autografts.

TABLE V

Reoperative Parathyroid Surgery



*1 Neither Positive

Project Description:

Part II. Thyroid Studies

Objectives:

The Surgery Branch provides surgical support for the Clinical Endocrinology Branch of the National Institute of Arthritis, Metabolism, and Digestive Diseases evaluating patients with thyroid cold nodules and thyroid neoplasms. The Surgery Branch is, itself, committed to the evaluation of patients with metastatic medullary carcinoma of the thyroid gland and provides a major service in providing screening for medullary carcinoma of the thyroid.

Methods Employed:

The Clinical Endocrinology Branch of the National Institute of Arthritis, Metabolism, and Digestive Diseases has recently published their experience with cold thyroid nodules. These patients are operated on by the Surgery Branch.

The Surgery Branch has been involved in two aspects of medullary carcinoma of the thyroid gland.

(1) In an effort to delineate those patients who have residual metastatic disease, basal thyrocalcitonin levels have been determined in the surgical service laboratories and in those patients whose levels have not been above normal, an attempt has been made to identify residual disease by peripheral vein determinations of pentagastrin-stimulated thyrocalcitonin levels.

(2) In addition, the Surgery Branch has collaborated with Dr. John Doppman to catheterize the central neck veins and hepatic veins during pentagastrin stimulation to determine thyrocalcitonin levels. On two occasions, clinically unsuspected disease has been revealed on neck catheterization to have elevated levels in the jugular veins. Both of these cases were proven, following modified radical neck dissection, to have positive metastatic deposits in the cervical node chain. These are the classic patients in which the modified radical approach has been employed with preservation of the contour of the neck by preservation of the sternomastoid muscle and resection of the internal jugular vein and all the cervical chain nodes.

Major Findings:

Thyrocalcitonin assay is now running as an integral part of the Branch and providing a major service to other sections within the hospital. No specific research protocols have been instituted.

Significance to Cancer Research and the Program of the Institute:

Major contribution is a service function to other departments. The medullary carcinoma cervical catheterization allows the identification of disease at an early state and will allow determination as to whether such biological markers can ever be used in the absence of clinical disease to provide a surgical setting where a patient may be rendered surgically-free of disease.

Proposed Course:

The Surgery Branch will continue their commitment as a service function to the Clinical Endocrinology Branch and will continue the studies of metastatic medullary carcinoma of the thyroid.

Project Description:

Part III. Reduction Mammoplasty

Objectives:

The collaborative study on male patients with gynecomastia in conjunction with the National Institute of Child Health and Human Development has been admitting approximately one patient per month for reduction mammoplasty. The National Institute of Child Health and Human Development undertakes an extensive work-up studying the hormonal aspects of these patients prior to surgery. The purpose of the reduction mammoplasty is to reduce the size of the breast toward a normal male contour and to obtain breast specimens for routine pathology examination and for analysis for hormone receptors.

Methods Employed:

Reduction mammoplasty is undertaken under general anesthesia in the operating suite of the Surgery Branch, National Cancer Institute. In patients with slight to moderate breast enlargement, reduction is done through a circumareolar incision removing most of the protruding fat and breast tissue. Some tissue is left underlying the nipple to prevent a concavity deformity. Patients with excessive mammary development have undergone one of several plastic surgery procedures which involve transposition of the nipples and areolae in addition to reduction of the breast mass.

Major Findings:

Following completion of the endocrinologic work-up, reduction mammoplasty is an essential feature of rehabilitation for this group of patients. All of the procedures have afforded adequate samples of breast tissue for study by the National Institute of Child Health and Human Development.

Significance to Cancer Research and the Program of the Institute:

While some of the patients operated have Klinefelter's syndrome and are thought to be at increased risk for the development of cancer, the majority of the patients are undergoing this procedure for cosmetic reasons. This study is undertaken primarily in the interest of supporting a study of another Institute in the Clinical Center.

Proposed Course:

The collaboration with the National Institute of Child Health and Human Development will be continued so long as a program of study for patients with gynecomastia is underway. Surgical techniques will be modified with further experience.

Project Description:

Part IV. Evaluation of the Countercurrent Exchange of Testosterone in the Spermatic Artery and Vein of Men with Infertility

Objectives:

Recent studies in the rat and monkey have demonstrated a higher concentration of testosterone in the spermatic artery than in the peripheral circulation. This concentration gradient results from a countercurrent transfer of testosterone to the artery from surrounding testicular veins. This study assesses the countercurrent exchange mechanism in normal men, men with infertility associated with varicocele, and men with idiopathic infertility.

Methods Employed:

Two groups of patients are being studied. A group of apparently normal males, ages 18-40, who are to undergo surgical procedures which require exposure of the spermatic cord are asked to volunteer for the study. A complete evaluation of reproductive function is performed. At the operation, blood is drawn from the femoral artery and vein and from the distal and proximal spermatic artery and vein. A second group of infertile men, participants in an ongoing comprehensive study of male infertility, who require an operative procedure for evaluation of infertility (such as testicular biopsy), are likewise asked to volunteer for the intraoperative blood sampling. This second group is to include patients with varicoceles and with idiopathic infertility.

The blood samples are assayed for testosterone, cortisol, FSH, LH, total protein, and urea nitrogen.

Major Findings:

At this time, preliminary data are available from 8 patients: 2 undergoing hydrocelectomy, 2 orchiectomy for breast cancer, and 4 varicocelectomy. In each case, the distal spermatic artery concentration of testosterone was higher than the proximal arterial concentration. Furthermore, in 6 of the 8 cases, the peripheral arterial testosterone concentration was lower than that measured in the proximal artery. No consistent differences in the levels of cortisol, LH, FSH, BUN, or total protein have been found. These preliminary results support the hypothesis that a countercurrent exchange mechanism for testosterone in the spermatic cord of man results in an increased arterial testosterone concentration in the testicular inflow.

Significance to Cancer Research and the Program of the Institute:

This study of testicular physiology in normal men and patients with infertility was designed in an attempt to produce new insights into the pathophysiology of infertility. The subjects of the study are men whose operative care is normally provided by the Surgery Branch as part of its commitment to make consultative service available to the Reproductive Research Branch of the NICHHD. The development of this collaborative study is an effort to make these services more productive, and the preliminary results have already demonstrated the existence in man of an exchange mechanism for testosterone in the spermatic cord.

Proposed Course:

The study will be continued to completion as outlined, to determine whether alterations in the spermatic countercurrent exchange of testosterone play a role in infertility secondary to varicoceles.

Project Description:

Part V. Nutritional Support

Objectives:

The Surgery Branch has provided a major service commitment to nutritional support within the National Institutes of Health. This consists of a Senior Investigator, three Clinical Associates, a total parenteral nutrition nurse, and laboratory and data analysis support. The object was to fill a badly needed service within the National Institutes of Health and try to develop some meaningful methods of both assessing the adequacy of parenteral nutrition and to develop specific, randomized protocols to examine the effectiveness of adjunctive nutritional support to primary modalities of surgery, chemotherapy, or radiotherapy in the treatment of cancer.

Methods Employed:

The service is a consultative service, and functions like other aspects of the consultative service of the Surgery Branch. Patients are seen on a referral basis and a decision is made by the team as to whether they would be most appropriately helped by total parenteral nutrition by vein or whether other methods of nutritional support are adequate. This service has not been aggressive about obtaining patients although large numbers of patients will benefit nutritionally. There seems little doubt that many patients within the National Institutes of Health remain chronically malnourished and could be helped by this service, but under the present system only the most severely malnourished are treated.

Major Findings:

The service commitment is illustrated in the accompanying table (Table VI).

TABLE VI

Total Parenteral Nutrition Statistics

April 4, 1977

Number of TPN patients	47
Number of therapy courses	52
Mean days per course	21
Total days of TPN	1125
Number of catheter insertions	65
Maximum days per course	126
Minimum days per course	0
Number of catheters for TPN	60
Maximum days of catheter	71
Minimum days of catheter	1
Mean days of TPN per catheter	18

Significance to Cancer Research and the Program of the Institute:

This is a major commitment of the Surgery Branch in terms of better management of patients with cancer. With the advent of total parenteral nutrition and the ability to maintain patients who would otherwise die of malnutrition during aggressive forms of cancer therapy, we now have an obligation to examine whether that therapy is indeed helpful in the management of the patient with cancer, or whether it is merely a method of either meaningless prolongation of life or makes no contribution to the cancer-bearing host.

Proposed Course:

This service commitment will continue and further randomized protocols will be developed.

Project Description:

Part VI. Ovarian Cancer

Objectives:

This Medicine Branch protocol, with which the Surgery Branch is collaborating, is designed to evaluate adjuvant treatment in selected patients with cancer of the ovary. The purpose of this study is to compare the usefulness of Melphalan chemotherapy and intra-abdominal instillation of radioactive phosphorus in resectable Stage II and poor prognosis Stage I patients, and to determine whether an improvement in survival will follow the addition of pelvic radiotherapy to standard surgical and chemotherapeutic treatment of incompletely resected Stage II ovarian carcinoma.

Methods Employed:

The standard surgical procedure will be total abdominal hysterectomy and salpingo-oophorectomy. When total removal of the tumor is not possible, as much disease as possible should be removed. An estimate of the bulk of disease at initial surgery and that remaining after surgery will be made.

Patients with certain stages and histological grades of ovarian carcinoma and no macroscopic residual ovarian carcinoma discovered at laparotomy will then be randomized to receive Melphalan or to receive radioactive chromic phosphate administered intraperitoneally. Patients with Stage II-B, II-C, or unresected residual disease will be randomized to receive in the postoperative period either Melphalan or Melphalan and pelvic radiotherapy.

Major Findings:

This program has just been initiated, and no data are currently available.

Significance to Cancer Research and the Program of the Institute:

This study will determine whether in selected patients Melphalan chemotherapy, intra-abdominal instillation of radioactive phosphorus, and pelvic radiotherapy can benefit patients with little or no macroscopic residual tumor.

Proposed Course:

These studies will be continued.

Publications:

None

In Press:

Gershengon, M.C., Beaven, M.A., McClung, M.R., Broadus, A.E., Chu, E.W., Brennan, M.F., and Robbins, J.C.: Elevated histaminase activity in needle aspiration biopsy of thyroid nodules for diagnosis of medullary carcinoma. N. Engl. J. Med.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 03801-07 SURG

PERIOD COVERED

July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Clinical Studies in Cancer Surgery

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	S. A. Rosenberg	Chief of Surgery, NCI	SURG	NCI
	and entire staff of	Surgery Branch, NCI	SURG	NCI
Other:	L. B. Thomas	Chief, Lab. of Pathology	LP	NCI
	W. Terry	Chief, Immunology Br.	I	NCI
	R. C. Young	Chief, Medicine Branch	M	NCI
	B. A. Chabner	Head, Biochem. Pharm. Sect.	LCHPH	NCI
	M. Lippman	Senior Investigator	M	NCI
	R. E. Johnson	Chief, Rad. Oncol. Br.	RO	NCI
	J. L. Doppman	Chief, Diag. Radiol. Dept.	DR	CC
	W. Schain	Psychologist	TRCC	NCI

COOPERATING UNITS (if any)

B. S. Bloom, Office of Planning and Evaluation, DHEW

LAB/BRANCH

Surgery Branch

SECTION

Office of the Chief

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
10.6	7.6	3.0

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The Surgery Branch has a variety of studies investigating innovative therapies for patients with malignant disease. The major emphasis of these studies is in the treatment of malignant melanoma, soft tissue sarcomas, and osteogenic sarcomas. New protocols for the treatment of rectal cancer have begun in the past year. The major emphasis in Surgery Branch cancer therapy is in adjunctive therapy with emphasis on the use of immunotherapy as a surgical adjuvant.

PHS-6040
(Rev. 10-76)

Project Description:

- Part I. Surgical Treatment of Malignant Melanoma
  - Part a. Natural History
  - Part b. Immunotherapy and Chemotherapy as Postsurgical Adjuvants in the Treatment of Malignant Melanoma
  - Part c. Evaluation of Staging Laparotomy, Iliac, Obturator, and Para-aortic Lymph Node Dissection in Patients with Melanoma
  - Part d. Studies on Intralesional Treatment of Cutaneous Melanoma Recurrences
  - Part e. Intralesional BCG as a Treatment for Patients with a Primary Malignant Melanoma
  - Part f. An Investigation of Human Melanoma for the Presence of Steroid Hormone Receptors
- Part II. Surgical Treatment of Sarcomas
  - Part a. Retrospective Sarcoma Study
  - Part b. Improved Treatment of Soft Tissue Sarcomas of the Extremities, Head and Neck and Trunk
  - Part c. Osteogenic Sarcomas
  - Part d. Epidemiologic Studies
  - Part e. Rehabilitation Studies
- Part III. The Use of High-Dose Methotrexate as a Preoperative Adjuvant in Patients with Squamous Carcinomas of the Head and Neck
- Part IV. Breast Cancer
  - Part a. Treatment of Primary Cancer
  - Part b. Treatment of Metastatic Breast Cancer
- Part V. Genitourinary Aspects of Cancer Management
  - Part a. Management of Massive Bladder Hemorrhage Secondary to Cyclophosphamide or Irradiation
  - Part b. Tumor Markers in Germ Cell Tumors of the Testis

Part c. Intensive Reductive Surgery and Chemotherapy for Metastatic Nonseminomatous Testicular Tumors

Part d. Innovative Techniques of Urinary Diversion in Patients with Pelvic Cancer

Part VI. Endoscopy

Part a. Laparoscopy

Part b. Colonoscopy

Part VII. Computer Applications to Research and Clinical Problems in the Surgery Branch

Part a. Randomized Protocols

Part b. Serum/Cell Data Base

Part c. Nutrition Studies

Part VIII. Colorectal Cancer

Part a. Pathogenesis Studies

Part b. Surgical Treatment of Rectal Cancer

Part IX. The Use of Thymosin as an Adjuvant Immunotherapy Treatment in Patients Receiving Chemotherapy for Oat Cell Carcinoma of the Lung and in Patients Receiving Radiation Therapy for Carcinoma of the Lung and Esophagus

Part X. Prospective Randomized Studies of Total Parenteral Nutrition as an Adjunct to Aggressive Chemotherapy

Part XI. Assessment of Quality of Life Change of Cancer Patients Undergoing Treatment in Clinical Trials

Project Description:

Part I. Surgical Treatment of Malignant Melanoma

Part a. Natural History

Objectives:

The purpose of this study was to review a 19 year experience in the Surgery Branch patients undergoing lymphadenectomy for malignant melanoma. We have concentrated particularly on correlations between Clark's level of invasion, thickness of the primary melanoma, status of the regional lymph nodes, and survival.

Methods Employed:

Records were reviewed of patients who underwent lymph node dissection in the Surgery Branch between 1955 and 1974. After excluding patients whose slides of the primary lesion were not available or inadequate, there remained 117 patients who had undergone lymphadenectomy, and for whom slides of the primary melanoma were considered adequate by the pathologists for determination of level of invasion. Sixty-one of the lymphadenectomies were considered "prophylactic", for clinically negative lymph nodes, and 57 lymph node dissections were considered "therapeutic" for clinically positive lymph nodes. One patient underwent two lymph node dissections for two separate primary melanomas.

Major Findings:

The preoperative assessment of the status of the regional lymph nodes was accurate 91% of the time when the surgeon felt the nodes were clinically positive, and accurate 79% of the time when the nodes were clinically negative. The 10 year survival in patients with 1-3 histologically positive nodes or no positive nodes was 50-55%, compared to a 25% 8 year survival in patients with 4 or more histologically positive nodes. Stepwise multivariate evaluation of prognostic factors indicated that the most important factor for predicting prognosis is the number of nodes histologically involved. Node palpability was the second most important factor because of its high correlation with number of nodes histologically involved. Site of melanoma was the third most important factor, as patients with extremity (upper or lower) melanoma had a better survival ($p=0.002$) than patients with axial melanoma (trunk or head and neck). Five years following lymphadenectomy there appeared to be substantial differences in survival according to differences in the level of invasion of the primary lesion; however, these differences were not nearly as pronounced 10 years following node dissection.

The division of melanoma thickness into <1.50 mm and >1.50 mm provided some prognostic discrimination at 5 years, but again the differences were not pronounced 10 years following node dissection. The thickness measurements were easier to determine than the level of invasion, and more reproducible on resubmission to the same pathologist. Four patients with melanoma thicknesses less than 0.76 mm had subsequent metastases, but these may represent inadequate sampling of the primary melanoma both in our series and in the four similar patients previously reported with such thin metastasizing melanomas.

Significance to Cancer Research and the Program of the Institute:

It is hoped that this additional information may be of help in determining prognosis for melanoma patients undergoing lymphadenectomy.

Proposed Course:

We plan to use this study as one of the bases for evaluation of the Surgery Branch approach to the treatment of malignant melanoma.

Project Description:

Part b. Immunotherapy and Chemotherapy as Postsurgical Adjuvants in the Treatment of Malignant Melanoma

Objectives:

The purpose of this study is to evaluate in a prospective randomized protocol the value of chemotherapy and immunotherapy in patients with Stage II malignant melanoma following local excision and regional lymph node dissection.

Methods Employed:

This protocol is being conducted in conjunction with the Medical Oncology and Immunology Branches. Eligible patients include those with Level IV melanomas greater than 2.25 mm thick, Level V lesions, all pathologic Stage II patients, and all patients with local recurrence of melanoma within 5 cm of the original primary melanoma. All patients receive wide local excision and regional lymph node dissection. Patients are then randomized to one of four arms. Patients randomized to observation alone are followed in the Surgery Branch. Patients randomized to chemotherapy receive 200 mg/m^2 of methyl-CCNU every six weeks. Patients randomized to receive BCG or BCG plus immunization with allogeneic neuraminidase-treated cells are followed in the Immunology Branch. Immunologic evaluation of patients is performed serially. To date, 109 patients have been entered into the above protocol. The Surgery Branch performs excisions and lymph node dissections on about one-half of all patients entering this protocol.

Major Findings:

Too early to draw conclusions.

Significance to Cancer Research and the Program of the Institute:

This protocol is designed to evaluate the role of immunotherapy and chemotherapy in the treatment of patients with malignant melanoma. These postoperative adjuvant treatments may improve the poor prognosis of patients with this diesase.

Proposed Course:

This study is being continued.

Project Description:

Part c. Evaluation of Staging Laparotomy, Iliac, Obturator, and Para-aortic Lymph Node Dissection in Patients with Melanoma

Objectives:

We have previously reported that approximately 13% of melanoma patients with metastases to the superficial groin are found to have metastases to the ipsilateral iliac and obturator lymph nodes. The purpose of the current study is to evaluate the relative efficacy of iliac and obturator lymph node dissection plus superficial inguinal lymph node dissection, compared to superficial inguinal lymph node dissection alone in the treatment of patients with melanoma metastatic to clinically negative superficial inguinal lymph nodes. In addition, we plan to evaluate the relative efficacy of para-aortic and deep inguinal lymph node dissection plus superficial inguinal lymph node dissection compared to superficial inguinal lymph node dissection alone in the treatment of patients with melanoma metastatic to clinically positive superficial inguinal lymph nodes.

Methods Employed:

Patients having lower extremity or truncal melanoma that are found to have pathologic involvement of the superficial inguinal lymph nodes will be randomized to one of two treatment groups, so they will or will not receive a deep inguinal lymph node dissection. To be eligible for randomization, the patient must have either pathological (microscopically confirmed) positivity in the superficial inguinal lymph nodes or a clinically positive examination confirmed by needle aspiration, or a history of having had at least one positive lymph node removed. All positivity shall have been confirmed by the NIH Laboratory of Pathology. All patients will have complete superficial lymph node dissection. Patients will be admitted for evaluation for this protocol within 3 months of superficial groin dissection. Patients with clinically positive superficial nodes will receive superficial inguinal dissection and will be randomized to receive either (1) no deep inguinal lymph node dissection, or (2) laparotomy, deep inguinal lymph node dissection, para-aortic lymph node dissection and needle biopsy of the liver. Patients with clinically negative, microscopically positive superficial inguinal nodes will also have received superficial inguinal dissection and will be randomized to receive either (1) no deep inguinal lymph node dissection, or (2) laparotomy, deep inguinal lymph node dissection, para-aortic lymph node biopsy (not dissection unless biopsy positive and liver negative for metastatic disease) and needle biopsy of the liver. Following postoperative convalescence, all patients will be eligible for entry into adjuvant programs for patients with Stage II malignant melanoma. The time of first appearance of subsequent recurrence of metastatic disease will be documented. Survival data will also be accumulated.

Major Findings:

Four patients have been randomized on this protocol to date. None of the patients has recurred.

Significance to Cancer Research and the Program of the Institute:

These studies are designed to answer the question of the efficacy of iliac, obturator, and para-aortic lymph node dissection in patients with melanoma metastatic to the superficial inguinal area. The answer to this question will be of considerable therapeutic importance in helping to determine the role of dissection of these lymph nodes in patients with melanoma metastatic to the superficial inguinal area. In addition, biologic information regarding the incidence of lymph node positivity in the iliac, obturator, para-aortic, and liver areas as a function of the degree of involvement in the superficial inguinal lymph nodes, will be forthcoming from this study. The incidence of para-aortic lymph node positivity is unknown to date because these lymph nodes have not been dissected in the past. Based on the ability of this lymph node dissection to help control patients with testis tumors metastatic to this area, this protocol has been designed along similar lines.

Proposed Course:

This study is being continued.

Project Description:

Part d. Studies on Intralesional Treatment of Cutaneous Melanoma Recurrences

Objectives:

To evaluate the role of BCG and dinitrochlorobenzene (DNCB) for the intralesional treatment of recurrent cutaneous malignant melanoma.

Methods Employed:

In an initial nonrandomized study on five patients, 82 lesions were injected with BCG (10 Tice organisms per injection), 179 lesions were injected with DNCB (50 micrograms), 35 lesions were injected with a combination of BCG and DNCB, 43 lesions were injected with acetone, the diluent for DNCB, and 59 lesions were inoculated with either saline, water, or other diluents. In a second study 17 patients have been randomized to receive only intralesional BCG or only intralesional DNCB. To be eligible for the randomized study, the patient must have had a malignant melanoma that was resected and had recurred, including multiple discrete deposits in the dermis or subcutaneous tissue. Each patient had undergone multiple surgical procedures culminating in the opinion of the attending physicians that the local disease could not be controlled by surgery. No patients with known visceral disease or hematogenous dissemination were included.

Each patient was then randomly placed into one of the two treatment groups - an intralesional BCG group and an intralesional DNCB group. Patients underwent intralesional injection of up to 20 lesions per round, at 4-6 week intervals.

Major Findings:

Both agents were able to destroy over 90% of the injected nodules although DNCB required two injections more frequently than did BCG in order to destroy individual nodules. Intradermal disease was more easily obliterated than subcutaneous disease with intralesional treatment with either agent. In the randomized study, one of eight patients in each group is currently free of evident disease. BCG injections regularly cause systemic symptoms and one patient almost died after a second round of BCG injections. DNCB has produced no systemic symptoms to date.

Significance to Cancer Research and the Program of the Institute:

We have been impressed with the ability of a nonreplicating agent to give equivalent regression rates as is observed after injection of BCG, a living agent, capable of causing regression of micrometastases in the Zbar guinea pig model, but associated with a high incidence of side effects. If DNCB is capable of producing the same clinical results as BCG, then it is also possible that the chemical agent might be substituted for BCG in studies on systemic immunotherapy as an adjuvant to treatment of solid tumors.

Proposed Course:

This study is being continued.

Project Description:

Part e. Intralesional BCG as a Treatment for Patients with a Primary Malignant Melanoma

Objectives:

This study is designed to determine whether treatment with intralesional BCG is capable of improving the prognosis of patients with primary malignant melanoma.

Methods Employed:

All patients having primary malignant melanomas have either punch or incisional biopsy. Those patients that have Clark's Level IV or V malignant melanoma are entered into a prospective, randomized trial that contains two treatment arms. The first treatment arm consists of immediate wide local excision with regional lymph node dissection. In the second treatment arm patients receive intralesional BCG followed two to four weeks later by wide local excision and regional lymph node dissection. Patients are then followed both clinically and with serial immunologic studies. To date, approximately 60 patients with primary malignant melanoma have been seen in the Surgery Branch and 25 patients have been entered into this treatment protocol. Thirteen have received intralesional BCG injection and 12 patients have received immediate surgery.

Major Findings:

Too early to draw conclusions.

Significance to Cancer Treatment and the Program of the Institute:

This study is designed to improve the prognosis and survival of patients with poor prognosis primary malignant melanoma using immunotherapy with intralesional BCG.

Proposed Course:

This study is being continued.

Project Description:

Part f. An Investigation of Human Melanoma for the Presence of Steroid Hormone Receptors

Objectives:

This study was designed to investigate the possibility that human melanoma contains steroid hormone receptor proteins; patients with estrogen receptors who have failed conventional therapy will be treated with additive estrogen therapy to determine the significance of the receptor protein.

Methods Employed:

Patients with melanoma have a portion of their tumor assayed for steriod hormone receptors using a specific competitive protein-binding assay. Pathologic control is obtained on all tumor samples.

Major Findings:

Thirty-five patients had tumor samples assayed for steroid hormone receptor activity. The tumor was from primary lesions in three patients, a soft tissue recurrence in 13, a metastatic lymph node in 17, a liver metastasis in one, and a pulmonary metastasis in one patient. Estrogen receptor activity was present in 16 of 35 patients, androgen receptor was found in 5 of 30 patients, progesterone receptor in 6 of 27 patients, and glucocorticoid receptor in 5 of 26 patients.

Significance to Cancer Research and the Program of the Institute:

The biologic behavior of melanoma suggests that there may be a subset of patients with a hormonally responsive tumor. This study has demonstrated that specific steroid hormone receptors are present in human melanoma. It is too early to state if the use of hormone receptor analyses will be useful in selecting a hormonally responsive subset of patients.

Proposed Course:

A larger number of melanoma tumor samples must be assayed to determine the incidence of receptor positivity and to make clinical correlations. Additional patients must be treated with hormonal therapy to determine its efficacy.

Project Description:

Part II. Surgical Treatment of Sarcomas

Part a. Retrospective Sarcoma Study

Objectives:

The objectives of this project were to review, verify, update, and analyze information on patients treated for sarcomas by the Surgery Branch from 1954 through 1974.

Methods Employed:

(1) Information on sarcoma patients had been initially collected and stored using the data forms and system developed in collaboration with the Division of Computer Research and Technology.
(2) During the past year the patient records were reviewed and the computer data file was corrected and brought up-to-date through December 1974.
(3) Corrected information was entered using WYLBUR.
(4) The tape master file was updated primarily by using the editing and updating programs generated by the DCRT Data Management System. Several additional special updates were written using IRS.
(5) Retrieval, display, and analysis of the sarcoma data was accomplished with IRS and a survival/recurrence/plot program developed by the Biostatistical Information Systems Unit.

Major Findings:

In this study 284 patients were classified into four categories - patients who:

(1) Received definitive surgical treatment for primary or persistent disease with no metastasis.
(2) Received definitive surgical treatment for locally recurrent disease with no metastasis.
(3) Presented with metastatic disease.
(4) Presented with primary or persistent disease with no metastasis who received no treatment or treatment other than surgery.

Table VII shows the distribution of patients by category and histologic diagnosis. Survival and recurrence data are currently being analyzed.

Significance to Cancer Research and the Program of the Institute:

This information constitutes a historical control for comparison with that obtained from the randomized protocols.

Proposed Course:

Analysis of the information will continue.

TABLE VII

Surgery Branch Experience with Soft Tissue and Bony Sarcomas

	(1)	(2)	(3)	(4)	Total
Fibrosarcoma	12	11	9	3	35
Liposarcoma	13	4	1	6	24
Osteosarcoma	32	2	21	6	61
Parosteal sarcoma	3				3
Chondrosarcoma	16	4	8	13	41
Neurofibrosarcoma	1	1			2
Leiomyosarcoma	3	1	3	2	9
Rhabdomyosarcoma	19	7	8	8	42
Mesenchymoma	3	2	1	2	8
Synovial cell sarcoma	3	2	8	2	15
Undifferentiated sarcoma	10	4	4	6	24
Other sarcoma	9	3	6	2	20
Total	124	41	69	50	284

Project Description:

Part b. Improved Treatment of Soft Tissue Sarcomas of the Extremities, Head and Neck and Trunk

Objectives:

To attempt to improve the treatment of patients with soft tissue sarcomas. In the treatment of soft tissue sarcomas of the extremities, the value of radical surgery, chemotherapy and immunotherapy are being evaluated. In the treatment of soft tissue sarcomas of the head and neck and trunk, the value of postoperative adjuvant chemo- and immunotherapy are being evaluated.

Methods Employed:

All patients with soft tissue sarcomas of the extremities with no evidence of metastatic disease are being randomized to one of three treatment arms. In one treatment arm, patients receive standard radical excisional surgery plus postoperative chemotherapy. In the second treatment arm, patients receive conservative surgery followed by high-dose radiation therapy and postoperative adjuvant chemotherapy. In the third treatment arm, patients receive conservative surgery, high-dose radiation therapy and postoperative chemotherapy and immunotherapy. Chemotherapy in all arms consists of adriamycin and cytoxan. Immunotherapy consists of the intravenous injection of Corynebacterium parvum.

Patients having soft tissue sarcomas of the head and neck or trunk without evidence of distant metastases are randomized to a treatment protocol consisting of two arms. One treatment arm receives surgery and adjuvant chemotherapy with adriamycin and cytoxan. The second treatment arm consists of surgery followed by adjuvant chemotherapy and immunotherapy.

All patients are extensively evaluated both clinically and in terms of immunologic competence. Patients are followed postoperatively with serial monitoring of immunologic, as well as clinical status.

Major Findings:

To date, 41 patients have been randomized in these treatment protocols. Thus far, there have been only 2 recurrences. One recurrence occurred in a patient receiving conservative surgery, radiation therapy and immunotherapy in the extremity protocol. The second recurrence occurred in a patient receiving chemotherapy alone in the head, neck and trunk protocol. By comparison with the extensive Surgery Branch historical control experience of 284 patients with soft tissue sarcomas, adjuvant immunotherapy appears to be having an effect in reducing the incidence of local recurrence and distant metastases. Of interest is the fact that most patients receiving radiotherapy and chemotherapy in combination have tolerated this combination extremely well. The reported difficulties with administering high-dose adriamycin and concomitant radiation therapy have not been seen. To date, both protocols, however, are too preliminary to draw conclusions regarding their relative therapeutic effectiveness of the radical vs. conservative surgical options in the extremity protocol and of the potential value of immunotherapy in the truncal protocol.

Significance to Cancer Research and the Program of the Institute:

This study is designed to improve the poor prognosis of patients with soft tissue sarcomas.

Proposed Course:

This study is being continued.

Project Description:

Part c. Osteogenic Sarcomas

Objectives:

This clinical protocol is designed to test the effectiveness of adjuvant chemotherapy and immunotherapy in the postoperative treatment of patients with osteogenic sarcomas.

Methods Employed:

All patients with osteogenic sarcomas without evidence of distant metastatic spread receive amputations in the Surgery Branch to achieve local control of their tumors. All patients then receive high-dose methotrexate with citrovorum rescue. This chemotherapy begins at 50 mg/kg and escalates to 250 mg/kg as tolerated. All patients are randomized to one of two treatment arms. One treatment arm receives high-dose methotrexate alone. The second group receives high-dose methotrexate plus weekly injections of BCG by Heaf gun scarification. All patients are extensively studied both clinically and in terms of their immunologic competence preoperatively and serially in the postoperative period.

Major Findings:

To date, 30 patients have been entered into this treatment protocol. We have not in these initial patients seen any difference between the use of adjuvant chemotherapy compared to our historical controls of surgery alone. The data are too preliminary to draw any conclusions regarding the relative effectiveness of BCG as a postoperative adjuvant, although no dramatic differences have been seen between these two groups.

Significance to Cancer Research and the Program of the Institute:

This study is designed to improve the treatment of patients with osteogenic sarcomas.

Proposed Course:

This study is continuing. We have recently changed the protocol to increase the escalation times of the methotrexate so as to give patients more methotrexate earlier in their postoperative course.

Project Description:

Part d. Epidemiologic Studies

Objectives:

In collaboration with the Epidemiology Branch of the National Cancer Institute special effort is being exerted toward the study of patients with bone and soft-part sarcoma. Initially charts on old patients with these diagnoses seen in the Clinical Center since 1953 were reviewed. In addition, new patients currently entered under specific sarcoma protocols have been interviewed in the clinic and hospital. The purpose of these studies is to uncover etiologic associations and risk factors that may be of importance in understanding the occurrence of this group of highly malignant but relatively rare tumors.

Methods Employed:

A computerized data set was developed to identify all patients in the group. Hospital records were then reviewed to extract specific demographic and other historical data. In patients currently under observation or treatment in the clinic or hospital, personal interviews were undertaken in addition to chart reviews.

Major Findings:

To date, 338 sarcoma patients have been identified. These represent 12 identifiable cell types. Three categories of information have been accumulated on these cell types. Host factors include predisposing disease, associated malignant and benign tumors, birth defects, and other factors. Overall in this category it is noted that 18% of the patients have some identifiable host factor. In the family history category, which includes the same subclassifications, 13% of the patients have identifiable family history associations. Relatively few of the patients have been identified with environmental factors.

Significance to Cancer Research and the Program of the Institute:

The findings of these epidemiologic studies will serve to confirm previous observations in the literature and will serve as the basis for further investigations. More detailed and carefully controlled research instruments must be developed in order to facilitate the studies.

Proposed Course:

Current studies will be expanded by means of prospective studies on new patients admitted to the Clinical Center. Interview techniques will be enlarged to obtain more specific information from all patients entering with a diagnosis of sarcoma as well as from old patients returning for follow-up examination. Following these pilot studies, further epidemiologic investigations using control groups for comparison will be considered.

Project Description:

Part e. Rehabilitation Studies

Objectives:

To investigate methods of improving the total course of rehabilitation in patients receiving amputations as major ablative surgery for osteogenic sarcoma of the lower extremities. To diminish the time required between surgery and functional use of a permanent prosthesis and to increase the quality and capacity of ambulation in all stages throughout the rehabilitation period.

Methods Employed:

Patients receiving above-the-knee amputations for osteosarcoma were chosen for investigation. A thorough review of the experience of major rehabilitation centers throughout the world was made. From this review and from preliminary experience at the NCI, rigid dressings (composed of plaster of paris) have been chosen as the best type of dressing for all stumps. Unclear, however, was whether immediate ambulation (24 hours post-surgery) or delayed but early ambulation (10 days to 2 weeks post-surgery at the time of suture removal) was superior. Also unclear was whether the necessary hardware for ambulation need be prepared by a prosthetist or could be prepared by a physical therapist.

Therefore, prior to surgery, patients have been prospectively randomized to receiving either a prosthesis that allows for immediate ambulation or for delayed ambulation. They have at the same time been randomized to have the prosthesis prepared by a prosthetist or by members of the Department of Physical Medicine at the NIH. An intense and thoughtful program of rehabilitation has been devised allowing for reasonably rigid comparison of multiple aspects of rehabilitation among the various groups.

Major Findings:

Both arms have been well tolerated and data are too preliminary to draw conclusions as to the relative effectiveness of these two approaches.

Significance to Cancer Research and the Program of the Institute:

The prognosis for prolonged survival in osteogenic sarcoma is still dismal. Still we are obligated to provide in those patients receiving major amputation for tumors the best chance for as normal, functional, and productive a life as is possible. The rehabilitation of patients who receive such amputations is, therefore, a vital part of the total care of patients treated at this Institute and is, therefore, a significant part of research relating to the patient and his tumor.

Proposed Course:

Continued research in rehabilitation of the amputee patient with cancer as outlined above. Possible extension of protocols to patients with other types of amputations. Continued provision of excellent quality prostheses for all eligible amputees and of programs for total rehabilitation of the amputee patient.

Project Description:

Part III. The Use of High-Dose Methotrexate as a Preoperative Adjuvant in Patients with Squamous Carcinoma of the Head and Neck

Objectives:

To develop a preoperative chemotherapy regimen in patients with squamous carcinoma of the head and neck that would fulfill the following criteria: (1) Be well tolerated with minimal hepatic or renal toxicity, (2) allow tumor resection within 1 to 2 weeks after the cessation of the chemotherapy without any increased pre- intra- or postoperative morbidity, (3) maintain serum methotrexate levels greater than 10^{-5} molar for a minimum of 36 hours, and (4) be compatible with other agents in a multiple drug preoperative regimen such as with bleomycin and cis-platinum.

Methods Employed:

Ten patients with biopsy-proven squamous carcinomas of the head and neck were given high-dose intravenous methotrexate 1500 mg/m^2 over 36 hours, preceded by a 50 mg/m^2 I.V. bolus, and followed by leucovorin rescue consisting of a bolus of 25 mg/m^2 I.V. over 15 minutes followed by leucovorin 200 mg/m^2 infused over 12 hours I.V., followed by leucovorin 25 mg/m^2 I.M. q 6 h for 6 doses. Daily CBC, platelets, and T cell levels were monitored. Methotrexate levels were determined at 1, 4, 12, 24, 36, 42, 48, 60, 72, and 84 hours post-infusion. Two cycles were given, the first beginning on day 1 of the regimen and the second beginning on day 8. The patients had surgical resection of the tumors on day 17 to 24. Liver enzymes and serum creatinine levels as well as 24-hour creatinine clearances were monitored at regular intervals for evidence of hepatic or renal toxicity.

Major Findings:

All patients tolerated two cycles of this regimen without untoward side effects that interdicted operation within 1 week following the second course of methotrexate. There was no increased mortality or postoperative morbidity. There was no significant myelosuppression, hepatic or renal toxicity. Serum methotrexate levels were maintained at 10^{-5} or greater molar for 36 hours in all patients.

Significance to Cancer Research and the Program of the Institute:

The major side effect with the 6-hour high-dose methotrexate pulse is renal toxicity, which may be secondary to the high levels of methotrexate that are attained (approximately 10^{-3} or 10^{-2} molar) during the infusion that predispose to precipitation of the methotrexate in the renal tubules during the clearance of the drug. With the 36-hour regimen and hydration and alkalinization the plasma methotrexate levels do not exceed 10^{-4}, thus minimizing precipitation in the renal tubules. In addition, in this study the leucovorin dosage used was much higher than that used traditionally with the 6-hour high-dose methotrexate pulse. There was negligible myelosuppression and renal toxicity despite the fact that 48-hour methotrexate levels were higher than with the high-dose 6-hour pulse. Therefore, this 36-hour methotrexate infusion for squamous cell carcinoma in elderly head and neck patients is well tolerated and compatible with operation shortly thereafter. This regimen probably would be compatible with other chemotherapeutic agents in a preoperative regimen package because of its negligible hepatorenal toxicities.

Proposed Course:

Trials are currently underway that evaluate the compatibility and adjuvant effect of this methotrexate regimen in combination with high-dose cis-platinum and bleomycin in patients with advanced but resectable head and neck squamous carcinomas.

Project Description:

Part IV. Breast Cancer

Part a. Treatment of Primary Cancer

Objectives:

Evaluation of postoperative chemotherapy and immunotherapy in the treatment of patients with cancer of the breast, metastatic to axillary lymph nodes, following mastectomy and axillary dissection.

Methods Employed:

The Medicine Branch, with the collaboration of the Surgery Branch and the Laboratory of Immunodiagnosis, is conducting Protocol BTS-135, Evaluation of a Combined Modality Approach to the Therapy of Mammary Carcinoma in Patients with T_1, 2, 3A, and 4B with N(+) Lesions. This is a protocol for adjuvant chemotherapy of surgically treated patients who have positive axillary nodes. Such patients are currently being randomized between chemotherapy with cytoxan, methotrexate and 5FU (CMF), vs. CMF with C. parvum, vs. observation (previously) or L-PAM (currently).

Major Findings:

The preliminary recurrence rates as presented in Table VIII are too early for evaluation. The lowest PHA stimulation values were found in the group treated with cytoxan, methotrexate and 5-Fluorouracil alone (Arm B), although none of the differences observed between the groups was statistically significant. In Arm B, the average PHA stimulation index (± SEM) was 46 ± 11. Arm C (CMF + C. parvum) had a mean stimulation index of 49 ± 15, and Arm A (observation and L-PAM) had a mean stimulation index of 50 ± 6.

Limited testing of cell-mediated cytotoxicity to long-term cultured breast cancer lines revealed no differences between the CMF arm and the observation/ L-PAM arm. All of these patients demonstrate skin test reactivity to recall antigens. In testing by the Laboratory of Immunodiagnosis, the percentage of rosette-forming cells was depressed during cytoxan, methotrexate, and 5-Fluorouracil therapy. This depression may not be as severe in patients receiving Corynebacterium parvum immunotherapy along with cytoxan, methotrexate, and 5-Fluorouracil.

Significance to Cancer Research and the Program of the Institute:

To date no information is available on the ability of C. parvum immunotherapy to affect the in vitro parameters and clinical course in patients receiving CMF chemotherapy. This study is an attempt to provide this information.

Proposed Course:

The treated patients will be followed for survival and further in vitro information. The Medicine Branch will determine whether new patient accrual will be pursued.

Project Description:

Part b. Treatment of Metastatic Breast Cancer

Objectives:

These studies are directed toward improving the treatment and diagnostic accuracy of predictive assays for patients with metastatic cancer. More reliable methods of identifying those patients who may be helped by endocrine ablation is also investigated.

Methods Employed:

The Surgery Branch has aided the Medicine Branch in a series of interlocking treatment protocols that have included palliative resections, endocrine ablation, and chemotherapy. These are illustrated by protocols BTS-122, BTS-123, and BTS-124. These protocols not only evaluate different chemotherapeutic regimens, but also the use of oophorectomy with adjuvant chemotherapy and endocrine manipulation in this group of patients with metastatic cancer of the

TABLE VIII

Clinical Status of Patients with Stage II Cancer of the Breast on the B135 Protocol

	Number of Patients	Number with Recurrent Disease (%)	Duration of Median Follow-up (Months)	Range of Follow-up (Months)
Observation	10	6 (60)[1]	9+	3+ - 36+
Observation to/or L-Phenylalanine mustard	12	3 (25)[2]	6	0+ - 10+
Cytoxan, Methotrexate, and 5-Fluorouracil	19	1 (5.2)[3]	12+	0+ - 31+
Cytoxan, Methotrexate and 5-Fluorouracil plus C. parvum	4	0	14+	6+ - 16+

[1] Relapsed at 3, 3, 7, 8, 8, and 11 months from diagnosis.

[2] Relapsed at 2, 9, and 15 months from diagnosis.

[3] Relapsed at 23 months from diagnosis after completing 20 cycles of Cytoxan, Methotrexate, and 5-Fluorouracil.

breast. Each of these studies is now closed to new patient accumulation; and BTS-122 has 88 randomized patients, BTS-123 has 27 randomized patients, and BTS-124 has 23 randomized patients. The Surgery Branch has provided multiple biopsies for the Medicine Branch laboratory involved for determination of a number of endocrine receptor proteins. These tissues provide the basis for evaluating the predictive value of endocrine receptors as markers. Protocol B151, The Prospective Use of Steroid Hormone Receptors in Predicting Response to Endocrine Manipulation in Breast Cancer, has been activated during the last year. A total of 7 patients have been randomized. Oophorectomy and oophorectomy with adrenalectomy have been performed by the Surgery Branch for the patients so randomized. All these patients have had their therapy directed by results obtained from hormone receptor protein assay results.

Major Findings:

Formal analysis of the first three protocols is being completed and data are being accumulated on the fourth-mentioned protocol.

Significance to Cancer Research and the Program of the Institute:

The information being accumulated will allow the application of the most appropriate therapy to the individual patient with metastatic breast cancer. This will be based on a better understanding of the individual biologic characteristics of the tumor. New treatment modalities are being evaluated in an attempt to prolong survival of patients with metastatic breast cancer.

Proposed Course:

It is planned to expand and further develop currently instituted protocols and studies with particular emphasis on the development of assays that would provide tumor markers and predictive evidence of response to therapy.

Project Description:

Part V. Genitourinary Aspects of Cancer Management

Part a. Management of Massive Bladder Hemorrhage Secondary to Cyclophosphamide or Irradiation

Objectives:

Severe acute hemorrhagic cystitis following administration of cyclophosphamide or radiation therapy for cancer has been relatively common among patients in the National Cancer Institute. Bleeding is occasionally massive and life-threatening, and despite the frequent use of multiple transfusions and other conventional modalities of treatment, the morbidity and mortality have been substantial. The purpose of this study is to evaluate the efficacy of local instillation of refined formaldehyde into the bladder to treat massive bladder hemorrhage.

Methods Employed:

The safety and efficacy of formaldehyde instillation into the bladder was first evaluated in animals. It was then applied to 12 patients with life-threatening hemorrhage refractory to conventional treatment.

Major Findings:

Cyclophosphamide induced the following changes in the bladder of man and experimental animals: (a) atypia of transitional cells with ulceration of the mucosa, (b) massive submucosal telangiectasia, (c) fibrosis of the detrusor muscle leading to diminished function of the bladder. These changes have been observed in patients suffering from cyclophosphamide cystitis, as well as radiation cystitis, and have been observed in mice and dogs experimentally. On the other hand, formaldehyde instillation into normal bladders of mice results in no significant changes detectable by biopsy. We have successfully utilized intravesical instillation of 200 ml of 5-10% refined formaldehyde in 12 patients with recurrent hematuria refractory to conventional therapy. Follow-up of these patients over 1-3 years has revealed no undesirable side effects. Three patients required a second instillation of formaldehyde.

Significance to Cancer Research and the Program of the Institute:

These studies have demonstrated an alternative therapy for intractable radiation or drug-induced cystitis with massive bleeding. This alternate modality of treatment does not require mutilating radical surgery which would leave the patient with a urinary diversion and loss of the bladder. The lack of complications of refined formaldehyde instillation and the effectiveness of hemostasis achieved have encouraged us to continue this program.

Proposed Course:

We plan to continue this study and follow the patients who have received this treatment with periodic bladder biopsy, cystometrogram, and renal function studies, to evaluate the long-term safety and effectiveness of this modality of treatment. A larger number of patients will be required for a better evaluation of the effectiveness and complications of intravesical instillation of refined formaldehyde.

Project Description:

Part b. Tumor Markers in Germ Cell Tumors of the Testis

Objectives:

Germ cell tumors of the testis have been shown to produce a variety of glycoproteins, including human chorionic gonadotropin (HCG), alpha fetoprotein (AFP), and carcinoembryonic antigen (CEA). The purpose of this study was to determine how frequently these tumor markers could be found in elevated levels in the peripheral circulation in patients with localized or regional disease; to assess the significance for prognosis of elevated markers prior to therapy; and to determine the value of serial marker assays as indicators of persistent or recurrent tumor after ablative therapy.

Methods Employed:

Serum samples obtained at regular intervals (usually weekly) on all patients were assayed for the beta subunit of HCG and for AFP by Dr. Thomas Waldmann, Metabolism Branch, NCI. CEA assays were performed on plasma samples by Hoffmann-LaRoche Laboratories as part of a collaborative study. Clinical data were obtained on 111 patients with clinical Stage I or II germ cell tumors of the testis at the Surgery Branch, NCI, and other cooperating institutions. Patients have been followed up to 40 months after therapy (mean 11.3 months).

Major Findings:

(1) Seminoma - HCG was elevated in only 2 of 21 patients, both with regional nodal metastases. AFP was never elevated in these patients. CEA was elevated in 4 of 7 but there was a poor correlation with the response to therapy, so that CEA was not an accurate marker of seminoma.

(2) Nonseminoma - Either HCG or AFP levels were elevated in 91% of the patients with clinically demonstrable nonseminomatous tumors. Among 24 patients who developed recurrent disease, 16 (67%) had elevated AFP or HCG at a time when recurrence was clinically undetectable. All patients free of tumor had normal levels of HCG or AFP; there were no false positive values. CEA was elevated in 33% of patients with seminoma and in 7% of patients with nonseminomatous tumor, but CEA levels did not correlate with the course of the disease. Determinations of HCG and AFP before lymphadenectomy decreased the error in clinical staging from 35% to 14%. Distant metastases developed in 67% (8/12) of Stage I and II patients with nonseminomatous tumor who had elevated levels of HCG or AFP prior to lymphadenectomy, but in only 4% (1/25) of patients when the HCG and AFP prior to lymphadenectomy were normal. The levels of HCG and AFP, but not CEA, indicated the true pathologic stage and the prognosis of these patients more accurately than did the conventional clinical parameters.

Significance to Cancer Research and the Program of the Institute:

This study has confirmed that circulating tumor markers can be found in elevated levels in the sera of most patients with active germ cell tumors of the testis. The accuracy of these assays in identifying even subclinical deposits of tumor, and the absence of false positive values, means that these assays can be a substantial aid in the early detection of testicular cancer, in providing a more accurate prognosis for these patients, and in monitoring their response to therapy.

Proposed Course:

The prospective study will continue to further define the accuracy of pretreatment marker levels in predicting recurrent and the value of serial marker determinations in detecting persistent or recurrent testicular cancer.

Project Description:

Part c. Intensive Reductive Surgery and Chemotherapy for Metastatic Nonseminomatous Testicular Tumors

Objectives:

The objective of this study is to determine whether surgical resection of all accessible tumor (debulking) combined with chemotherapy can prolong the response rate in and increase the survival rate of selected patients with advanced metastatic germ cell tumors of the testis, compared to chemotherapy alone.

Methods Employed:

Patients with suspected testicular germ cell tumors will have confirmation of the diagnosis by inguinal exploration of the testicle with radical orchiectomy when indicated by the presence of an intratesticular mass. If this procedure was performed prior to referral, confirmation of the histology will be required by review of the specimen by the Department of Pathology, NIH. Only patients with nonseminomatous germ cell tumors of the testis will be eligible. Nonseminomatous tumors are those with histologic elements of embryonal carcinoma, infantile embryonal carcinoma or teratoma with or without seminoma and choriocarcinoma.

Patients will undergo a thorough medical evaluation to document the extent of the tumor. Only patients with metastases beyond the infradiaphragmatic lymph nodes, or with extensive involvement of these nodes, will be included in this study. These patients must have metastases in areas accessible to surgical resection (retroperitoneum, lung), although the presence of additional inaccessible metastases (liver, mediastinum) will not preclude participation.

Major Findings:

In an initial pilot study in 4 patients we demonstrated the low morbidity of the debulking procedure in patients with bulky disseminated germ cell tumor of the testis. These patients tolerated the intensive surgery followed or preceded by intensive chemotherapy rather well. So far 6 patients have been entered into the prospective randomized protocol. Three have been randomized to chemotherapy. Of 3 patients randomized to surgery, one died of massive pulmonary metastases and respiratory insufficiency before having the opportunity to perform any surgery. One patient underwent a debulking procedure and about 1200 gm of tumor tissue was removed. This patient is currently receiving intensive chemotherapy. Obviously data are too preliminary to draw any conclusions concerning the effectiveness of debulking surgery.

Significance to Cancer Research and the Program of the Institute:

There is no prospective randomized study available to assess the role of debulking surgery. This study is designed to assess the role of reductive surgery and possibly improve the prognosis and survival of these patients by utilizing intensive combined therapeutic modalities.

Proposed Course:

This study is being continued.

Project Description:

Part d. Innovative Techniques of Urinary Diversion in Patients with Pelvic Cancer

Objectives:

The ileal conduit has been the most widely used method of supravesical urinary diversion in patients with pelvic malignancy. However, conventional ileal conduits have not been without complications. Most series report a 30-70% incidence of urinary complications following ileal conduits, and approximately 20% of ileal conduits need surgical revision. These procedures are often difficult due to adhesions and chronic enteritis from previous surgical procedures and/or pelvic irradiation. Pelvic irradiation induces an ischemic enteritis of the distal small intestine which has settled or adhered to the pelvic floor making the ileum especially unsuitable for use as a conduit. The objective of this study is to design a technique of high jejunal urinary drainage in patients with pelvic exenteration and failure of the ileal conduit. This technique is preferable to ileal conduit revision, because it avoids tedious dissection in a field which has had prior surgery and/or irradiation.

Methods Employed:

The jejunum which is fixed in the upper abdomen by the ligament of Treitz is mobilized and interposed between the renal pelvis and skin. This proximal renal pelvis anastomosis is preferable to avoid subsequent possible ureteral obstruction secondary to radiation ureteritis, recurrent tumor, or retroperitoneal fibrosis. Thus the entire procedure is performed on more viable tissue rather than the devitalized pelvis.

Major Findings:

This operation has been performed on 4 patients with failure of ileal urinary diversion following pelvic exenteration for invasive pelvic malignancy. All 4 patients had either multiple pelvic surgical procedures and/or pelvic irradiation. Interposition of jejunum between the proximal ureters or renal pelvises and skin was performed with satisfactory results in all 4 patients. In patients with pelvic exenteration and failure of ileal urinary diversion this technique offers the following advantages over the conventional revision of the ileal conduit: (1) eliminates use of irradiated small intestine which is subject to fistula formation and/or obstruction, (2) avoids the settling of the urinary conduit in the devitalized pelvic space with potential adhesions, fistula formation, or obstruction, (3) proximal jejunal loop is away from area of potential tumor recurrence and field of previous or future radiotherapy, (4) eliminates the extensive dissection of the adherent ileal conduit and subsequent risk of avascular necrosis, and (5) bypasses the irradiated fibrotic distal ureters.

Significance to Cancer Research and the Program of the Institute:

Two hundred ninety-six pelvic exenterations in patients with pelvic malignancies have been performed at the Surgery Branch of the NCI. This technique of relieving the obstructive uropathy that leads to renal failure has a significant role in improving the survival of these patients.

Proposed Course:

This technique as well as other innovative surgical techniques such as interposing a jejunal loop between the ureters and the bladder are being continued. Patients having these operations are being followed for the long-term results.

Project Description:

Part VI. Endoscopy

Part a. Laparoscopy

Objectives:

To define the proper role of this minimally invasive diagnostic endoscopic examination in patients with tumors under study in the Surgery Branch.

Diagnostic laparoscopy and translaparoscopic tubal ligation are available as a consultative service for appropriate patients.

Methods Employed:

Laparoscopy is performed in the operating room under general anesthesia using the newly purchased Wolf endoscopic equipment. Double puncture technique is utilized. In addition to careful inspection of all appropriate visceral surfaces each examination includes a targeted or random biopsy of the liver and cytologic washings.

Major Findings:

Eleven patients have undergone laparoscopy; 10 as part of their staging evaluation for the Surgery Branch rectal cancer protocol.

Two patients were found at laparoscopy to have histologically verified hepatic metastatic disease (one rectal cancer, one melanoma). Only one of these was suspected preoperatively on the basis of abnormal liver chemistry, scan evidence or physical examination.

Of the remaining 9 patients examined, 5 had subsequent laparotomy. In each instance careful exploration of the peritoneal cavity was carried out. In one of these 5 instances a small focus of hepatic metastatic disease was found.

Significance to Cancer Research and the Program of the Institute:

Laparoscopy incurs less patient morbidity, mortality, and expense than does laparotomy. By permitting better assessment of tissues only poorly evaluable by present clinical, chemical, roentgenographic, and radioisotopic scanning techniques appropriate utilization of this minimally invasive endoscopic procedure should contribute to improving the overall quality of care for certain cancer patients.

Proposed Course:

Continuing efforts to determine the sensitivity and accuracy of diagnostic laparoscopy are planned in conjunction with both the rectal cancer and newly activated Stage II melanoma protocols.

Project Description:

Part b. Colonoscopy

Objectives:

Fiberoptic endoscopy of the lower GI tract is available as a consultative service function of the Surgery Branch. The utility of this procedure from both the diagnostic and therapeutic point of view of colon lesions is well established. Its use as a screening device for colorectal malignancy is being explored.

Methods Employed:

Olympus colonoscopes of both the short (110 cm) and long (190 cm) type are available. Techniques for intracolonic photography, biopsy, snare polypectomy or obtaining cytological specimens are currently in use.

Major Findings:

Snare polypectomy has become the established method of treating pedunculated and sessile colonic polyps. The savings in patient mortality, morbidity, and cost has been well established. Five polypectomies were performed without complications.

Assessment of extent and severity of inflammatory bowel disease by multiple mucosal biopsies from each portion of the colon has improved the management of this complex disease. Colonoscopy with biopsy allows earlier diagnosis of ulcerative colitis and Crohn's colitis when patients are symptomatic but when sigmoidoscopic and barium enema examination may yet be entirely normal. The extent of disease and its distribution (total or segmental inflammation) can be determined more accurately by colonoscopic multiple biopsy and visual inspection than by barium enema examination. The presence of malignant degeneration or dysplastic epithelium that usually accompanies malignancy in a patient with long standing inflammatory bowel disease can be detected accurately through multiple endoscopic biopsy. Finally, colonoscopy may assist in the differentiation of ulcerative colitis from Crohn's colitis. Four patients underwent complete colonoscopy with multiple biopsy to assist in the management of inflammatory bowel disease.

Preoperative evaluation of patients prior to colorectal surgery assists in detection of additional intracolonic lesions and may establish an unequivocal diagnosis by biopsy of the primary lesion. It also assists the radiologist by directly inspecting confusing areas of the colon seen on barium enema. Ten patients underwent colonoscopy for these indications. Many of these patients were being evaluated for treatment in the Surgery Branch rectal cancer protocol.

Significance to Cancer Research and the Program of the Institute:

Direct visual access to and ability to histologically sample tissues from the lower gastrointestinal tract offer excellent opportunities for earlier diagnosis of colon malignancy. Initial efforts to test available colonoscopic detection methods involve the screening of high risk populations. Those now under study are patients with ulcerative colities and those with a prior colorectal malignancy. Clinical trials to establish the role of serial colonoscopic examinations in these two patient populations are underway.

Proposed Course:

Our objective is to devise a rapid, safe, self-contained screening device for colorectal cancer which a technician or nurse can operate. A collaborative project with the A. D. Little Company, Inc., Boston, MA, is being developed.

Project Description:

Part VII. Computer Applications to Research and Clinical Problems in the Surgery Branch

Part a. Randomized Protocols

Objectives:

A data base information management system is being maintained for the randomized protocols currently in progress in the Surgery Branch. The objectives for this system are to:

(1) Store minimal but extremely accurate information on each patient.
(2) Permit the information to be easily keyentered, verified, corrected, retrieved, and analyzed.
(3) Use data collection forms and entry procedures which provide maximum flexibility; i.e., where items may be added or deleted from protocols or field sizes changed as the study progresses.
(4) Use information retrieval techniques which are minimally or easily modified when the data base structure changes.

(5) Produce routine summaries of the information so that it is available and helpful in the ongoing follow-up of each patient.
(6) Make use of existing utility, statistical, and life table analysis programs for the generation of survival rates, recurrence rates, disease-free intervals, and other statistics describing the characteristics of the protocol populations.

Methods Employed:

The randomized protocol system incorporates the following methods:

(1) Data collection is performed by a research nurse who is thoroughly familiar with and actively participates in the implementation of the protocols. This helps ensure accuracy and consistency of the information.
(2) The data forms are designed in three parts: Evaluation, Treatment, and Follow-up. Each part contains several sections of data elements to be collected as specified by the protocol. The forms are stored in a WYLBUR data set so they may be easily revised as necessary.
(3) Data is entered using WYLBUR with data entry formats corresponding to sections on the forms.
(4) The data base is updated using Inquiry and Reporting Svstem (IRS) and DCRT utility procedures.
(5) All data is 100% verified by visually comparing a listing of the information after it is entered against the source data forms.
(6) Data base entries may be corrected directly using WYLBUR text editing commands.
(7) A record of information in the data base corresponds to a section of information on a data form and is uniquely identifiable for each patient.
(8) IRS is used to produce routine reports as well as to reformat information for the more complicated analytical programs which are generally written in FORTRAN.

Major Findings:

Information for five randomized protocols has been maintained by the system during the past year. The following table gives statistics on information in the data base as of April 1977:

	Protocol Patients		Nonprotocol Patients*	
Disease Protocol	No. of Patients	No. of Records	No. of Patients	No. of Records
SB-M1 Malignant Melanoma	23	278	34	166
SB-S1 Soft Tissue Sarcoma of Extremities	23	414	18	90
SB-S2 Soft Tissue Sarcoma of Head, Neck, Trunk	13	190	0	0
SB-S3 Osteogenic Sarcoma	28	785	7	39
SB-R1 Rectal Cancer	5	56	13	85
Total	92	1723	72	380

*Patients not qualified for the protocol whose disease courses are also being followed.

Significance to Cancer Research and the Program of the Institute:

This system enables the Surgery Branch to maintain up-to-date records of individuals being followed in a readily accessible and useful form. The data accumulated by this project will be used to generate statistics concerning recurrence and survival in the individual diseases that are being treated.

Proposed Course:

As new randomized protocols are developed, the system will be extended to include the additional information.

Project Description:

Part b. Serum/Cell Data Base

Objectives:

The objective of this project is to maintain a data base of blood component and immune evaluation records which are well characterized clinically and are readily accessible.

Methods Employed:

The system operates with the following characteristics:

(1) The initial inventory records are designed to contain:
  (a) Biographical patient information used to identify the individuals (name, age, sex, etc.).
  (b) Coded descriptions of each patient's primary tumor by site, histology, and extent using the Manual of Tumor Nomenclature and Coding (MOTNAC).
  (c) Serum identification number with corresponding patient identification number, date of acquisition, and the quantity of serum and lymphocyte vials cryopreserved.
  (d) Assay data generated from specific serum samples. The information recorded includes WBC, differential, percentages of T cells and B cells, lymphocyte response to PHA, MLC, PPD, and thymosin stimulation, and skin test results.

(2) Data is entered using the NIH/DCRT WYLBUR system.

(3) The editing and updating programs are written in COBOL and the information is maintained on magnetic tapes.

(4) Retrieval and display programs are written using the Inquiry and Reporting System (IRS).

Major Findings:

By April 1977, 9084 separate blood drawings had been made from 4955 patients. A total of 27,594 vials of serum have been frozen in liquid nitrogen, an average of three vials per drawing. In addition, 5936 vials of lymphocytes have been cryopreserved.

The data base also contains 5081 cell count records; 2025 PHA, MLC, and PPD stimulation records; 124 skin test records; 3427 thymosin stimulation records; 100 thymosin protocol treatment and evaluation records; and 71 thymosin protocol follow-up records.

Significance to Cancer Research and the Program of the Institute:

The development of a blood component inventory system is of central importance in longitudinal serologic studies of cancer patients.

Proposed Course:

These studies will continue. The serum and lymphocyte bank will continue to expand.

Project Description:

Part c. Nutrition Studies

Objectives:

A data base information management system is being maintained for studies conducted by the Metabolism Section of the Surgery Branch. These include Total Parenteral Nutrition, Hyperparathyroid, Medullary Cancer of the Thyroid, and Glucose Tolerance Test. The objectives of the system are the same as those described in Part a.

Methods Employed:

The Nutrition Studies System has been designed using the same methods as those for the Randomized Protocol System. Data collection forms have been designed to meet the specific needs of each study. Additional features of the system include:

(1) A table look-up program written in FORTRAN to identify and produce daily totals of the components of the solutions administered in the Total Parenteral Nutrition study. These totals are then stored in the data base.
(2) Programs which use the clinical and hematology laboratory values stored in the Clinical Information Utility System. These values are combined with nutritional intake values to produce a report of the daily balance of fluids, nitrogen, and electrolytes.
(3) Programs which use the Integrated Plotting Package routines developed by DCRT. They produce graphs of nutritional intake and output parameters over a period of time.

Major Findings:

The following table shows the content of the data base as of April 1977:

Study	Patients	Records
Total Parenteral Nutrition	50*	7,639
Hyperparathyroid	24	743
Medullary Cancer of Thyroid	3	309
Glucose Tolerance Test	19	96
Nutrition and Tumor Growth	-	1,304
Total	96	10,091

*Includes 6 patients in a cooperative protocol with the Pediatric Oncology Branch for nutritional studies in children.

Significance to Cancer Research and the Program of the Institute:

There are many aspects of intravenous feeding, as an adjunct to medical and surgical care, in which exact requirements are not yet known. Accurate recording and storage of experimental data with subsequent analysis aids the effort to determine the requirements for various nutrients and the efficacy of infusions.

Proposed Course:

Data collection and anlysis will continue on the current studies.

Project Description:

Part VIII. Colorectal Cancer

Part a. Pathogenesis Studies

Objectives:

Epidemiologic evidence suggests that gut transit time may be an important variable in the pathogenesis of colorectal cancer. Efforts are currently being initiated to quantitate gut transit time in patients with colorectal tumors and to compare it to gut transit time in patients with nongastrointestinal tract malignancy.

Methods Employed:

The method of Hinton, using ingested radiopaque markers, is utilized to quantitate gut transit time.

Major Findings:

Only one patient has been studied thus far, hence no meaningful data are as yet available.

Significance to Cancer Research and the Program of the Institute:

Age and the presence of colon polyps are the only two variables which show a positive correlation with the propensity to develop colorectal cancer. Should gut transit time, for either genetic or environmental reasons, prove to be an important variable, a measurable parameter with predictive potential will have been recognized. Enhanced understanding of the pathogenesis of the tumor as well as possible population screening for the propensity to develop colorectal cancer are both potential gains.

Proposed Course:

Data will be collected and evaluated as planned.

Project Description:

Part b. Surgical Treatment of Rectal Cancer

Objectives:

To evaluate in a randomized, prospective fashion the role of electrofulguration alone and in combination with immunotherapy in the definitive treatment of rectal cancer. To assess the sensitivity and accuracy of preoperative laparoscopy in determining those patients who present with Stage D (disseminated) rectal cancer.

Methods Employed:

In May 1976, the Surgery Branch activated a three-armed study designed to compare electrofulguration alone or electrofulguration plus adjuvant BCG immunotherapy with abdominoperineal resection.

Major Findings:

To date 21 patients have been evaluated for this study. Seven are currently on protocol; abdominoperineal resection (3), electrofulguration alone (3), electrofulguration plus BCG (1).

Accrual has been disappointingly slow, yet not unexpected given the lack of an established referral pattern due to the paucity of previous Surgery Branch interest in this tumor and the ambient feeling in the community that this neoplasm is being well treated by time-tested surgical techniques.

Significance to Cancer Research and the Program of the Institute:

Colorectal cancer is the commonest visceral malignancy in man and the third most common cancer killer. Those patients with disease confined to the low rectum will all undergo abdominoperineal resection. This procedure carries a 5-10% operative mortality, a 50% probability of significant operative morbidity, the prospect of a permanent colostomy with its attendant emotional and psychosocial liability, and still only cures about 40% of the patients so treated. By comparison, the several noncontrolled published experiences with electrofulguration report no mortality, significantly less morbidity, no need for colostomy, and survival rates at least comparable to those of abdominoperineal resection.

If it can be shown in a prospective, appropriately controlled study that electrofulguration either alone or with adjuvant immunotherapy is at least as good as or better than abdominoperineal resection, a significant contribution to the management of these patients will have been made.

Proposed Course:

Patient accrual to this clinical program is being actively pursued.

Project Description:

Part IX: The Use of Thymosin as an Adjuvant Immunotherapy Treatment in Patients Receiving Chemotherapy for Oat Cell Carcinoma of the Lung and in Patients Receiving Radiation Therapy for Carcinoma of the Lung and Esophagus

Objectives:

Previous *in vitro* studies in this laboratory showed that lymphocytes from cancer patients receiving radiation and chemotherapy for carcinoma show an increase in the percentage of T cells when incubated with thymosin, a calf thymus gland extract. To test whether these findings reflect an ability of thymosin to have a similar effect on T cell levels *in vivo* in such patients, studies were designed to use thymosin in patients receiving high-dose chemotherapy for oat cell carcinoma of the lung at the Washington, DC, Veterans Administration Hospital and in patients receiving palliative radiation therapy for squamous carcinoma of the lung or esophagus at the Washington Hospital Center and the Howard University Medical Center. At the VA Hospital, patients are randomized to receive thymosin or placebo every other day during induction chemotherapy for a total of 6 weeks. Patients receiving radiation therapy at the Howard University Medical Center and the Washington Hospital Center are also randomized to receive thymosin or placebo.

Methods Employed:

The patients are randomized in a double blind prospective fashion to receive either placebo, thymosin 20 or thymosin 60 mg/m^2 injected subcutaneously. T cell levels with and without thymosin *in vitro* incubation are performed weekly. Evaluation of the response of each patient in the chemotherapy study is made at the end of 6 weeks after completion of induction of chemotherapy consisting of CCNU, cytoxan, and methotrexate. Thymosin administration is terminated at the end of this interval. The patients then receive no treatment for 6 weeks, are re-evaluated, and randomized to one of two other maintenance chemotherapy regimens. In the radiation therapy study, patients again have weekly laboratory studies performed during the course of radiation therapy and thymosin is given every other day for the period of radiation therapy.

Major Findings:

Sixty patients have now been entered into the oat cell carcinoma study at the VA Hospital. There are approximately 20 patients in each arm of the study. The in vitro data is currently undergoing analysis, and preliminarily indicate that patients receiving the higher dose of thymosin maintain total T cell levels slightly higher and for a longer period of time than patients receiving the placebo or the lower dose of thymosin, but statistical analysis has not verified this yet. Preliminary life table analyses also show that the patients receiving the higher dose of thymosin tend to survive longer than those receiving the lower dose or those receiving placebo, but again these differences are not statistically significant. Over 20 patients have been enrolled in the radiation therapy-thymosin study at the Washington Hospital Center and the Howard University Medical Center. Data from this study have not been analyzed since the number of patients is still too small to provide any meaningful conclusions.

Significance to Cancer Research and the Program of the Institute:

If thymosin is protective against the immunosuppressive effects of high-dose chemotherapy and radiation therapy, it may improve clinical course by reducing the incidence of infections or increasing tumor control rates.

Proposed Course:

Analysis of the preliminary study at the VA Hospital has led to the initiatio of a more intense chemotherapy induction study with randomization of patients to thymosin 60 mg/m^2 or placebo and deletion of the lower dose of thymosin. The studies in patients receiving radiation therapy will continue until a sufficient number of patients has been entered to merit analysis for effects of thymosin.

Project Description:

Part X. Prospective Randomized Studies of Total Parenteral Nutrition as an Adjunct to Aggressive Chemotherapy

Objectives:

Total parenteral nutrition is now being widely employed in cancer-bearing patients. While retrospective information suggests that this is advantageous to the patient, it is hard to separate the effects of total parenteral nutrition from aggressive care and from responses due solely to the aggressive therapy and consequent well-being of the patient. The objectives of such studies are to firmly delineate whether nutritional support has any role to play in the aggressive management of cancer-bearing patients.

Methods Employed:

Our initial study is with the Pediatric Oncology Branch attempting to approach the very aggressive regimens for metastatic osteosarcoma, Ewing's sarcoma, and rhabdomyosarcoma. These protocols have an approximately 20% therapy-related mortality. Long-term survival is 0 to 10% for osteosarcoma. All of these patients can expect to lose approximately 20% of their body weight during the therapeutic regimen.

In addition, characterization of the impact of nutrition on some aspects of chemotherapeutic tolerance, efficacy and preservation of lean tissue mass are also being assessed.

Major Findings:

Major findings are summarized in Table IX.

Significance to Cancer Research and the Program of the Institute:

Should total parenteral nutrition be shown to be of any effect in this highly-aggressive regimen, then it would justify moving it to much more effective therapy in other diseases which are similarly accompanied by considerable host-nutritional depletion.

Proposed Course:

More numbers will be required.

Project Description:

Part XI. Assessment of Quality of Life Change of Cancer Patients Undergoing Treatment in Clinical Trials

Objectives:

To provide the practicing physician with data that describe the economic, sociologic, and psychologic cost of different treatment plans. This assessment of quality of life change produced by cancer treatment is to be applicable generally to surgical, radiation, or chemotherapeutic regimens. Its initial application is in patients in the Surgery Branch extremity sarcoma protocol.

Methods Employed:

The assessment instrument took advantage of available questionnaires, as well as newly constructed scales. The instrument assessed the patient's socioeconomic status, economic condition (at any one time), mood state, change in behavior, social adjustment, sense of helplessness, response to treatment, and measure of vomiting. Scales constructed for this study included measures of socioeconomic and economic state, as well as response to treatment. A treatment trauma scale which ranks the particular procedures patients in different arms of the extremity sarcoma protocol must undergo is also used. This allows the patient to individually assess the suffering induced by his or her own treatment regimen in comparison to many other treatments common to all patients.

Major Findings:

A prospective study is being planned for all patients to be entered into the extremity sarcoma protocol. The retrospective study now in progress is to obtain data plus test our assessment instrument.

TABLE IX

Total Parenteral Nutrition Prospective Randomized Protocol for Patients with Metastatic Disease

	Chemotherapy	No TPN	A/D*	TPN	A/D
Metastatic Osteosarcoma	ADR,MTX,CTX VCR,DTIC,CF,PAM	1	1/0	2	2/0
Metastatic Ewing's Sarcoma	CTX,VCR,DTIC,ADR	2	0/2	1	0/1
Metastatic Rhabdomyosarcoma	CTX,ADR,VCR	1	0/1	1	0/1
Total as of 4/15/77		4	1/3	4	2/2
Follow-up (0→6 mo.)					

*A/D = Alive/Dead

Significance to Cancer Research and the Program of the Institute:

Unless major advances regarding the basic biology of cancer are made, treatment regimens are unlikely to produce striking differences in survival. Therefore, of several regimens that produce nonsignificant differences in survival statistics, the regimen which allows the best quality of life should be selected. The significance of this project is that it will measure the cost of various treatment regimens.

Proposed Course:

This study will be continued during the next year.

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 03807-03 SURG

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Immunoevaluation of Patients with Cancer

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	P. B. Chretien	Assistant Chief, Surg. Br.	SURG NCI
Other:	N. H. Goldberg	Clinical Associate	SURG NCI
	D. N. Weingrad	Clinical Associate	SURG NCI
	S. D. Lipson	Clinical Associate	SURG NCI
	W. Matthews	Chemist	SURG NCI

COOPERATING UNITS (if any)

LAB/BRANCH
Surgery Branch

SECTION
Tumor Immunology Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
4.5	3.0	1.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Data that show correlations of peripheral blood thymus dependent lymphocyte (T cell) levels as determined by in vitro E rosette formation and in vivo cell-mediated immunity give importance to the development of objective means of determining T cell levels. We have developed a technique for determining T cell levels by cytofluorographic analysis utilizing glutaraldehyde fixation of the lymphocytes that correlates with levels determined by light-microscope counting. Cytofluorographic display of lymphocytes from patients receiving radiation therapy and chemotherapy show patterns differing from normals or untreated cancer patients. Studies are in progress to determine the functional classification of these lymphocyte subpopulations and their correlation with clinical course and response to therapy. Recent studies demonstrate that cyclic nucleotide levels in human peripheral blood lymphocytes parallel T cell levels. Preliminary studies in our laboratory confirm this correlation. Studies in progress are designed to determine the usefulness of this assay as a correlate of cellular immunity and the host-tumor relationship. Particular emphasis will be placed on correlations of lymphocyte cyclic nucleotide levels and response to therapy in cancer patients.

PHS-6040
(Rev. 10-76)

Project Description:

Part I: The Use of Cytofluorographic Analysis to Determine E Rosette Levels and to Identify Abnormal Lymphocyte Subpopulations in Patients Receiving Chemo- Immuno- or Radiation Therapy

Part II: Measurement of Human Peripheral Blood Lymphocyte Cyclic Nucleotide Levels: Correlation with T Cell Levels and Immune Response

Project Description:

Part I: The Use of Cytofluorographic Analysis to Determine E Rosette Levels and to Identify Abnormal Lymphocyte Subpopulations in Patients Receiving Chemo- Immuno- or Radiation Therapy

Objectives:

In a previous study in this laboratory we automated the counting of E rosettes in normal volunteers using cytofluorographic analysis. When the technique was applied in a study to determine the E rosette levels of patients undergoing mediastinal radiation therapy for carcinoma of the lung and breast, the results did not correlate with light-microscope counting. Investigation of this problem revealed a subpopulation of lymphocytes in the irradiated patients that interfered with the accurate quantitation of the E rosettes. To further investigate this finding, we interfaced our Cytofluorograf with a Nuclear Data computer to obtain a three-dimensional picture of the cell populations and for better characterization of them. In addition, the computer provided a memory bank which allowed holding the information generated by the fluorescing lymphocytes and later analysis by a number of ways not otherwise possible. The objectives of this study were (1) to interface the Cytofluorograf with the computer in an attempt to measure blood levels of E rosettes in patients receiving irradiation, and (2) to try to better characterize the subpopulation of cells in these patients and investigate the prevalence of similar populations in patients receiving chemotherapy or immunotherapy.

Methods Employed:

Using the Cytofluorograf interfaced with a Nuclear Data 4420, we are currently characterizing the lymphocyte subpopulations of patients receiving chemo- immuno- and radiation therapy. Lymphocytes are separated on a Ficoll-Hypaque gradient and stained with acridine orange dye to allow discrimination by the Cytofluorograf on the basis of red and green fluorescence as well as light scatter. When the cell populations have been adequately characterized we will separate them with a cell sorter and examine them cytologically and functionally. The E rosettes formed by the lymphocytes of these patients will be measured using an acridine orange dye to stain the cells and using the fixative glutaraldehyde to maintain the stability of the rosettes. With the interfaced computer we can get a three-dimensional picture of the cell population and can more easily exclude the abnormal subpopulations from our counting process. In order to further separate the populations of lymphocytes, abnormal cells, and E rosettes, we are radiolabelling the sheep red blood cells with fluorescein using several different methods, one being direct conjugation of fluorescein and red cells and a second the use of rabbit anti-sheep red blood cell globulin that has been conjugated with fluorescein.

Major Findings:

Thus far the lymphocytes from patients receiving radiation therapy appear to have an abnormal population of lymphocytes. Smears confirm that these are not monocytes or cellular debris. A new computer program that will be in use soon will allow increased definition of this population and cell sorter analysis will allow further definition of the subpopulation.

Significance to Cancer Research and the Program of the Institute:

The significance of these findings is two-fold: (1) Automation of E rosette and cell determinations is an extremely valuable and time-saving technique. It will reduce variability using the assays read on the light-microscope and will allow the work to be done more efficiently and with the expenditure of less time. (2) The subpopulation of lymphocytes identified in these patients may be significant if the levels correlate with clinical course and/or response to chemo- or immuno- or radiation therapy.

Proposed Course:

Horizontal studies on patients during treatment will be done to correlate the level of the lymphocyte subpopulation with clinical course. Automated counting of E rosettes in patients receiving radio-, chemo-, or immunotherapy constitutes the major goal of the study.

Project Description:

Part II: Measurement of Human Peripheral Blood Lymphocyte Cyclic Nucleotide Levels: Correlation with T Cell Levels and Immune Response

Objectives:

There have been many reports in the past few years demonstrating the significance of intracellular levels of lymphocyte cyclic nucleotides in relation to their physiologic function. Phytohemagglutinin and other plant mitogens have been shown to alter lymphocyte cyclic AMP and cyclic GMP levels. More recent studies have demonstrated that levels of intracellular cyclic nucleotides in human peripheral blood lymphocytes reflected the immune status of patients as compared to T cell levels.

If the intracellular levels of cyclic AMP in human peripheral blood lymphocytes from patients with tumors at various stages with different histologies do in fact correlate with other primary parameters of cellular immunity, then measurement might perhaps provide a sensitive indicator of the effects of various therapies in altering cellular immunity. The objective of this study was to explore this hypothesis by determining the cyclic AMP levels in lymphocytes from patients with various histologies and stages of tumors undergoing radiotherapy as well as normal controls and correlate *in vitro* T cells and cyclic AMP levels.

Methods Employed:

Cyclic nucleotide and E rosette levels were determined in blood specimens from a group of 18 patients with tumors of the mediastinum and pelvis who were undergoing radiation therapy as well as 3 normal adults. Peripheral blood lymphocytes were isolated on a Ficoll-Hypaque gradient from blood heparinized with preservative-free heparin. T cell levels were quantitated by the percent lymphocytes forming spontaneous rosettes when mixed with sheep erythrocytes. Sextuplicate samples were prepared with T cell determinations and the tubes coded and read blindly by the same person. Cyclic AMP levels were determined on the spun supernatant of a lymphocyte suspension at 15 (10^6) cells/ml. The cells were first frozen in a dry ice-ethanol bath, thawed in a water bath at 37°C, and spun in a microcentrifuge. A double antibody radioimmunoassay for cyclic AMP was used to determine the cyclic AMP levels.

Major Findings:

From this preliminary study with a limited number of patients, it was determined that there was a correlation between the mean T cell level and the level of cyclic AMP. The correlation coefficient was approximately 0.4. Because of the small numbers, no effort was made to further separate the groups by histology and degree of treatment that had been previously done. However, analysis of further samples might show significant correlations within groups.

Significance to Cancer Research and the Program of the Institute:

Major preliminary findings so far suggest a correlation between cyclic AMP levels and T cell levels. This may provide a sensitive indicator to monitor immunotherapy and therapies affecting the immune response.

Proposed Course:

The findings of this study suggest the need for further collection of samples to determine if there are significant differences between groups of patients when separated by tumor histology, degree of therapy, and stage of disease. The potential use of this parameter for monitoring patients receiving immunotherapy with thymosin and other immunotherapeutic agents will be investigated.

Publications:

Silverman, N.A., Alexander, J.C., Jr., Hollinshead, A.C., and Chretien, P.B.: Correlation of tumor burden with *in vitro* lymphocyte reactivity and antibodies

to herpesvirus tumor-associated antigens in head and neck squamous carcinoma. Cancer 37:135-140, 1976.

Silverman, N.A., Alexander, J.C., Jr., Potvin, C., and Chretien, P.B.: In vitro lymphocyte reactivity and T cell levels in patients with melanoma: Correlations with clinical and pathological stage. Surgery 79:332-339, 1976.

Goldberg, N.H., Kenady, D.E., Super, B.S., and Chretien, P.B.: A technique for determining E rosette levels by cytofluorographic analysis. J. Immunol. Methods 12:9-17, 1976.

Hollinshead, A.C., Chretien, P.B., Lee, O.B., Tarpley, J.L., Kerney, S.E., Silverman, N.A., and Alexander, J.C.: In vivo and in vitro measurements of the relationship of human squamous carcinomas to herpes simplex virus tumor-associated antigens. Cancer Res. 36:821-828, 1976.

Tarpley, J.L., Twomey, P.L., Catalona, W.J., and Chretien, P.B.: Suppression of cellular immunity by anesthesia and operation. J. Surg. Res. 22:195-201, 1977.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 03809-03 SURG

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)
In Vitro Responsiveness to Thymosin of Lymphocytes from Patients with Solid Malignancies

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	P. B. Chretien	Assistant Chief, Surg. Br.	SURG NCI
Other:	D. N. Weingrad	Clinical Associate	SURG NCI
	W. Matthews	Biochemist	SURG NCI
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COOPERATING UNITS (if any)

LAB/BRANCH
Surgery Branch

SECTION
Tumor Immunology Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
4.5	2.5	2.0

CHECK APPROPRIATE BOX(ES)
☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

That thymosin, an extract of fetal calf thymus, improves in vitro and in vivo cellular immune responses in children with thymic deficiencies forms a rationale for determination of the effects of thymosin in vitro on T cell levels in patients with solid malignancies. Tumor-bearing patients with solid malignancies, patients receiving radiation therapy to head and neck, mediastinal, and pelvic portals, patients clinically cured of solid malignancies, and normal controls all show an inverse relationship between initial T cell levels and the in vitro response to thymosin: With progressively lower initial T cell levels the increase after incubation with thymosin increased progressively. As a group, the greatest increase occurred in patients receiving radiation therapy for mediastinal malignancies. The data constitute a basis for a determination of the effects of thymosin on cell-mediated responses and clinical course in patients with solid malignancies. The demonstrations that murine thymocytes exposed to thymosin have increases in cyclic GMP levels form the basis for current attempts to correlate in vitro response to thymosin and cGMP levels in human lymphocytes.

Project Description:

Part I: In Vitro Effect of Thymosin on T Cell Levels During Radiation Therapy: Correlations with Radiation Portal and Dose, Tumor Histology, and Initial T Cell Levels

Part II: In Vitro Effect of Thymosin on T Cell Levels in Tumor-Bearing and Clinically Cured Cancer Patients

Part III: In Vitro Effect of Thymosin on Intracellular Levels of Human Peripheral Blood Lymphocyte Cyclic Nucleotide Levels in Normal Volunteers and Patients Undergoing Radiation Therapy: Correlation with Initial T Cell Levels

Project Description:

Part I: In Vitro Effect of Thymosin on T Cell Levels During Radiation Therapy: Correlations with Radiation Portal and Dose, Tumor Histology, and Initial T Cell Levels

Objectives:

Recently, thymosin, an extract of fetal calf thymus, improved cellular immune responses in children with thymic deficiencies. The patients had low thymus-derived lymphocyte (T cell) levels which increased after incubation *in vitro* with thymosin. After treatment with thymosin, their circulating blood T cell levels increased and did not increase further after incubation with thymosin. These correlations suggest that if cancer patients have impaired cellular immunity that is secondary to deficiencies of factors vital for maturation of T cell precursors, thymosin may be similarly effective in increasing T cell levels and other parameters of cellular immunity.

If the thymus gland is the principal source of factors that lead to maturation of T cell precursors, irradiation of the mediastinal region may cause a decline in cellular immune response due to deleterious effects of irradiation on this function of the gland. If this mechanism is a major contributor to the decline in cellular immunity during mediastinal irradiation, then T cell levels in blood specimens from patients during mediastinal irradiation may increase after incubation with thymosin as occurred in children with thymic deficiencies.

The objective of this study was to explore these hypotheses by determining the comparative effects of thymosin *in vitro* on T cell levels in cancer patients receiving radiotherapy via mediastinal, head and neck, and pelvic portals.

Methods Employed:

The effect of thymosin *in vitro* on percent T cells was determined in 388 blood specimens from patients with head and neck, mediastinal, and pelvic malignancies during radiation therapy, in 94 untreated patients with these malignancies, and 277 normal adults. All patients had clinically localized tumors that were encompassed by the radiation portal. Peripheral blood T cell levels were quantitated by the determination of the percent of lymphocytes forming spontaneous rosettes when mixed with sheep erythrocytes. Triplicate samples were prepared for T cell determinations and 3 additional samples were prepared in an identical fashion with the addition to each tube of .1 ml of phosphate-buffered saline containing 100 μg of thymosin fraction 5 provided by Dr. Allan Goldstein. The tubes were coated so as to prevent identification of the blood donor or of the tubes containing thymosin by the person performing the T cell determinations. The effect of thymosin was determined by subtracting the mean of the triplicate samples without thymosin from the mean of the triplicate samples with thymosin.

Major Findings:

After incubation with thymosin, the mean T cell level in normals increased significantly ($p < .005$). In untreated patients with head and neck and pelvic malignancies the levels also increased significantly, but did not differ from the increase in normals. In untreated patients with mediastinal malignancies, however, the increase was not significant. Mean T cell levels in patients with head and neck and pelvic malignancies during irradiation did not change significantly after incubation with thymosin, and in pelvic malignancies the change was less than untreated patients ($p < .02$). However, in patients with mediastinal malignancies during irradiation the mean level increased ($p < .001$) and the increase was greater than in normals ($p < .05$). An additional analysis was done to ascertain whether the effect of thymosin correlated with tumor histology in patients receiving radiation therapy. Since all head and neck malignancies were squamous carcinomas, the mediastinal and pelvic malignancies were subdivided into squamous and nonsquamous carcinomas. There was no significant difference in the change in T cell levels after incubation with thymosin between those with squamous and those with nonsquamous carcinomas. This analysis shows that the significantly increased mean T cell level in patients with mediastinal malignancies did not correlate with tumor histology. Determinations of the relation between cumulative radiation dose and change in T cell levels showed no correlation in any of the groups studied. On the other hand, regression analysis showed a correlation between initial percent T cell levels and the effects of thymosin in all groups studied. There was a significant inverse relation between initial T cell levels and T cell levels after incubation with thymosin.

Significance to Cancer Research and the Program of the Institute:

The major finding in this study is that thymosin *in vitro* increases the percent T cell levels in both normals and cancer patients and the effect varies inversely with the initial T cell level. Furthermore, in patients receiving radiation therapy the increase in patients with mediastinal malignancies for a given T cell level was greater than in patients with pelvic malignancies, and as a group was greater than in patients with head and neck malignancies. If the data from the treatment of children with thymic deficiency apply, the data suggest that thymosin may increase T cell levels in patients with low T cell levels as a population. Thus, thymosin may be a useful immunoadjuvant in patients with low T cell levels associated with tumor burden or secondary to effects of immune suppressive therapy. The singular effects on T cell levels in patients with mediastinal malignancies during radiation therapy form a rationale for the determination of the effects of thymosin in combination with radiation therapy in patients with mediastinal malignancies.

Proposed Course:

The findings in this study constitute the basis for a prospective randomized study to determine the effects of thymosin in combination with radiation therapy in patients with carcinomas of the lung and esophagus localized to the thorax. Additional in vitro studies will be conducted that will further characterize the lymphocyte subpopulation accounting for the differences seen with the effects of thymosin in these patients.

Project Description:

Part II. In Vitro Effect of Thymosin on T Cell Levels in Tumor-Bearing and Clinically Cured Cancer Patients

Objectives:

The demonstrations that children with thymic deficiencies improve clinically after treatment with thymosin and manifest increase in T cell levels and other parameters of cell-mediated immunity suggest a possible application of this agent in the treatment of patients with solid malignancies and suppressed cell-mediated immunity. In the children with thymic deficiencies, response to thymosin treatment could be predicted by the in vitro response of lymphocytes to thymosin: A rise in T cell levels as determined by rosette formation with sheep red blood cells was predictive of a response to thymosin in vivo. This correlation formed the basis for a screen of tumor-bearing patients with solid malignancies and patients clinically cured of these tumors.

Methods Employed:

The effect of thymosin in vitro on percent T cells was determined in over 200 patients with clinically localized malignancies, 127 patients with no evidence of recurrence 5 or more years after tumor resection, and in 277 normals. The tumors represented were squamous carcinomas of the head and neck region, lung, esophagus, and cervix; adenocarcinomas of the lung, breast, and rectum; melanomas; and sarcomas. Patients with evidence of vital organ dysfunction or a history of previous malignancy were excluded. Peripheral blood T cell levels were quantitated by determination of the percent of lymphocytes forming spontaneous rosettes when mixed with sheep erythrocytes. Identical samples were also prepared with the addition of 100 μg of thymosin fraction 5 provided by Dr. Allan Goldstein. All samples were performed in triplicate and were coded and randomized so as to prevent identification of the donor by the person performing the T cell determinations. The effect of thymosin was defined as the mean of triplicate samples without thymosin minus the mean of triplicate samples incubated with thymosin.

Major Findings:

In normals, tumor-bearing patients, and patients clinically cured of malignant tumors by surgery, T cell levels after incubation with thymosin showed an inverse relationship with initial T cell levels. With progressively lower T cell levels, there was a progressively greater increase in the T cell levels after incubation with thymosin as determined by regression analysis. There were no remarkable correlations with tumor burden or histology. The slopes of the regressions derived from the effect of thymosin on tumor-bearing and clinically cured patients were identical and both were less than the slope of the regression derived from normals.

Significance to Cancer Research and the Program of the Institute:

The study shows that the in vitro effect of thymosin on T cell levels is related to the initial T cell level. This relationship suggests a potential value of the agent in elevating T cell levels in cancer patients with low T cell levels.

Proposed Course:

The findings of this study provide a rational basis for the determination of the effects of thymosin on cell-mediated immunity in patients with suppressed responses. Additional investigations will be required to determine whether this effect of thymosin occurs during general anesthesia and surgery and during the administration of immunosuppressive chemotherapy. If so, trials of the beneficial effect on cellular immune responses and course after cancer therapy of thymosin would appear warranted.

Project Description:

Part III: In Vitro Effect of Thymosin on Intracellular Levels of Human Peripheral Blood Lymphocyte Cyclic Nucleotide Levels in Normal Volunteers and Patients Undergoing Radiation Therapy: Correlation with Initial T Cell Levels

Objectives:

Demonstration that children with thymic deficiencies improve clinically after treatment with thymosin manifesting increases in T cell levels and other parameters of cell-mediated immunity suggests a possible application of this agent in the treatment of patients with solid malignancies and suppressed cell-mediated immunity. In the children with thymic deficiencies response to thymosin treatment could be predicted by the in vitro response of lymphocytes to thymosin. A rise in T cell levels determined by rosette formation with sheep red blood cells was predictive of response to thymosin in vivo. A screen of tumor-bearing patients with solid malignancies and patients clinically cured of these tumors has shown that thymosin effects significant increases in their

E rosette levels. The most marked effect was demonstrated in those patients receiving mediastinal irradiation. Animal work reveals that murine thymocytes exposed to thymosin had significant increases in the cyclic GMP level. These studies suggested that there might in fact be a correlation between the elevation in the T lymphocyte level after incubation with thymosin and the alteration in cyclic GMP following thymosin incubation.

Methods Employed:

The effect of thymosin *in vitro* on percent T cells and cyclic GMP and AMP levels was determined in 15 patients receiving pelvic and mediastinal irradiation and 3 normal subjects. The tumors represented were squamous cell carcinomas of the cervix, lung, esophagus; adenocarcinoma of the lung, breast and rectum; Hodgkin's disease; and metastatic testicular tumors. Patients with evidence of vital organ dysfunction or history of previous malignancy were excluded. Peripheral blood was collected in preservative-free heparin and the lymphocytes separated on a Ficoll-Hypaque gradient. Peripheral blood T cell levels were quantitated by determination of the percent lymphocytes forming spontaneous rosettes mixed with sheep erythrocytes. Identical samples were prepared with the addition of 100 μg of thymosin fraction 5 provided by Dr. Allan Goldstein. All samples were performed in sextuplicate, coded and randomized so as to prevent identification of the donor by the person performing the T cell determinations. The effect of thymosin was defined as the mean of sextuplicate samples without thymosin compared to the mean of sextuplicate samples incubated with thymosin. Cyclic GMP levels were determined on the spun, frozen-thawed supernatant of a 15 million cell/ml lymphocyte suspension. The determination of cyclic GMP was performed on triplicates using a specific radioimmune assay for cyclic GMP.

Major Findings:

The results of this work are in an extremely preliminary state. However, it has been determined that cyclic GMP levels do not correlate in any observable way with the T cell levels, although preliminary data suggest a correlation with cyclic AMP levels.

Significance to Cancer Research and the Program of the Institute:

A goal of this study is to demonstrate a correlation of the *in vitro* effect of thymosin on T cell levels and cyclic GMP levels. This relationship suggests the potential value of the cyclic GMP assay in accurately determining those agents which affect the immune response as well as those patients who might benefit most from treatment with thymosin.

Proposed Course:

The findings of this study are of a very preliminary nature, but encourage the continued investigation in further patients. Additional samples will be collected from both tumor-bearing patients undergoing radiotherapy and normal controls. The potential use of cGMP determinations to monitor patients during their course of immunotherapy with thymosin will be investigated.

Publications:

Kenady, D.E., Chretien, P.B., Potvin, C., and Simon, R.M.: Thymosin reconstitution of T cell deficits *in vitro* in cancer patients. *Cancer* 39:575-580, 1977.

Kenady, D.E., Chretien, P.B., Potvin, C., and Simon, R.M.: Effect of thymosin *in vitro* on T cell levels during radiation therapy: Correlations with radiation portal and initial T cell levels. *Cancer* 39:642-652, 1977.

Kenady, D.E., Potvin, C., Simon, R.M., and Chretien, P.B.: *In vitro* effect of thymosin on T-cell levels in cancer patients receiving radiation therapy. In Chirigos, M.A. (Ed.): *Control of Neoplasia by Modulation of the Immune System*. New York, Raven Press, 1977, pp. 305-313.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 03810-03 SURG

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Studies on the Circulating Glycoproteins of Cancer Patients

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	P. B. Chretien	Assistant Chief, Surg. Br.	SURG NCI
Other:	N. H. Goldberg	Clinical Associate	SURG NCI
	D. M. Crnic	Clinical Associate	SURG NCI

COOPERATING UNITS (if any)

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LAB/BRANCH
Surgery Branch

SECTION
Tumor Immunology Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS: 5.0	PROFESSIONAL: 2.5	OTHER: 2.5

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Serum carcinoembryonic antigen (CEA) levels in healthy nonsmokers and smokers show a progressive increase with age in each group and a consistently higher level in smokers. The results show that the currently used upper limit of normal for this assay is applicable only to nonsmokers of approximately 40 years of age. These findings may increase the utility of the assay as a marker for tumor presence in malignancies associated with elevations of CEA levels. CEA levels in head and neck cancer are elevated only in the presence of clinically manifest disease, thus diminishing the value of this assay as a tumor marker in such patients. Serum carbohydrates and serum glycoproteins show correlations with tumor burden in a variety of solid malignancies. In addition, certain carbohydrates and glycoproteins show an inverse relationship with in vitro lymphocyte reactivity. The studies indicate that additional investigations are warranted to determine the value of these moieties as a marker of tumor burden and correlate of immune reactivity in cancer patients.

Project Description:

Part I: Effect of Age and Cigarette Smoking on Carcinoembryonic Antigen Levels

Part II: Carcinoembryonic Antigen Levels in Head and Neck Cancer

Part III: Serum Glycoprotein Carbohydrate Levels in Patients with Solid Malignancies

Project Description:

Part I: Effect of Age and Cigarette Smoking on Carcinoembryonic Antigen Levels

Objectives:

After elevated levels of carcinoembryonic antigen (CEA) were found in patients with carcinoma of the colon, it was speculated that CEA was a tumor-specific antigen that would be of value for the identification of patients with carcinoma of the colon and a means of detecting tumor recurrence after treatment. Subsequently, elevated CEA levels were found in patients with carcinomas of the pancreas, lung, stomach, breast, and head and neck region. Furthermore, elevated CEA levels were found in patients with benign diseases such as chronic bronchitis, emphysema, ulcerative colitis, regional enteritis, and alcoholic cirrhosis. Such elevations of CEA levels in nonmalignant diseases limit the usefulness of the antigen as a marker of tumor presence and for monitoring of tumor response to treatment. The most common factor shown to be associated with elevations of CEA levels is cigarette smoking. Previous studies have shown a 15% incidence of elevated CEA levels in normals with a smoking history greater than 15 cigarettes a day compared to only 1.8% incidence in age-matched nonsmokers. The high prevalence of cigarette smoking makes it the most important known factor that may diminish the value of CEA as a tumor marker. The object of this study was to quantitate the effect on CEA levels of cigarette smoking in healthy smokers and determine the effect on CEA levels of cessation of cigarette smoking.

Methods Employed:

Healthy volunteers who were currently smoking cigarettes were studied. Those known to have active acute or chronic diseases and those with a history of malignancy were excluded. Healthy nonsmokers were also solicited and similarly screened. Specimens were obtained prior to cessation of smoking in those chronic smokers who ceased smoking and also obtained at 1, 3, and 6 months from those who maintained abstinence from smoking. CEA assay was performed by the Hansen Z gel technique.

Major Findings:

The mean CEA levels of nonsmokers was 1.9 ng/ml. The upper limit of "normal" for CEA levels was fixed at 2 standard deviations above the mean of nonsmokers

(4.94 ng/ml). CEA levels were determined to the nearest .1 ng, thus levels of 5.0 ng/ml or greater were considered elevated. Six of 122 (5%) nonsmokers had elevated CEA levels, a distribution identical to determination of CEA levels on plasma in nonsmokers, although both the mean and variability of serum CEA levels were greater than plasma CEA levels determined on the same blood tested both as serum and plasma. The mean CEA level of 154 smokers was 2.7 ng/ml and 18 (12%) had elevated levels. The mean CEA level of smokers was .8 ng/ml higher than that of nonsmokers ($p < .001$) and the number of smokers with abnormal CEA levels was greater ($p < .05$). In both smokers and nonsmokers, CEA levels decreased with age. Among nonsmokers the median CEA level rose from 1.4 ng/ml in the third decade to 1.9 ng/ml in those greater than 50 years of age. Among smokers, the median CEA level rose from .9 ng/ml in the third decade to 2.9 ng/ml in those over 50 years of age. Regression of CEA levels on age in both smokers and nonsmokers showed a significant correlation ($p < .01$). CEA levels appeared equivalently related to age in smokers and nonsmokers. Regression of CEA levels on pack-years of cigarette consumption showed a significant correlation ($p < .02$). Age and pack-years of smoking history also correlated ($p < .001$). Seventy-six of the 154 smokers ceased smoking for at least one month and were restudied at 1, 3, and 6 months afterwards, or until they resumed smoking. One month after cessation of smoking, their CEA level was lower than while smoking, but was higher than nonsmokers. Of those studied 3 months after cessation of smoking, their CEA level did not differ from nonsmokers. Thus, all elevated CEA levels in smokers declined to within the range of nonsmokers within 3 months among those who ceased smoking for this interval of time.

Significance to Cancer Research and the Program of the Institute:

The data indicate that smoking has a predictable influence on CEA levels and elevates serum CEA levels approximately .8 ng/ml, regardless of age or amount of cigarettes consumed. Also, after cessation of smoking, CEA levels declined to levels similar to nonsmokers within a 3 month period. This observation is important for the clinical evaluation of CEA levels in former smokers. CEA levels obtained greater than 3 months after the cessation of smoking appear to be evaluable independent of prior smoking history. The correlations of CEA levels with age in both smokers and nonsmokers indicate that a reappraisal of the diseases associated with elevated CEA levels using an upper limit of normal that considers the influence of age and smoking habits may invalidate some of the positive correlations previously reported. Also, consideration of age and smoking history may improve the correlations between CEA levels and tumor burden and thus extend the clinical utility of CEA as a marker of tumor presence.

Proposed Course:

Studies now in progress compare CEA levels with serum levels of glycoproteins to determine whether serum levels of several glycoproteins evaluated as a group may serve as a better index of tumor burden than those glycoproteins elevated in cancer patients when considered singly. An integral part of this analysis is the correlation of CEA levels and the levels of other glycoproteins being studied with immune reactivity and in vitro correlates of cellular immunity in the patients studied.

Project Description:

Part II. Carcinoembryonic Antigen Levels in Head and Neck Cancer

Objectives:

In previous studies on patients with carcinomas of the lung and head and neck region, elevations of CEA levels have been reported. However, because of the epidemiological association with cigarette consumption and these tumors, the data may not reflect tumor burden but rather may be due to the effects of cigarette consumption. This hypothesis derives from our findings of significant elevations in CEA levels associated with chronic cigarette consumption (Part I). Because of the need for serum markers of tumor burden in monitoring host response to tumor treatment and earlier detection of subclinical residual disease, this study was conducted to correlate CEA levels with tumor burden in patients with head and neck carcinomas using as controls normal subjects of similar age and with similar levels of cigarette consumption.

Methods Employed:

Over 400 patients with squamous carcinomas of the head and neck region were studied. One hundred seventy-six were tumor-bearing at the time of testing, and of these, 31 were studied sequentially at varying intervals from 2 months to 4 years after surgical resection. An additional 23 patients were studied at intervals during definitive radiation therapy. A final group of 147 patients who were tumor-free for greater than 3 years after treatment were studied. The CEA levels were determined on serum samples using the Hansen Z gel assay. The controls were 276 healthy volunteers selected by age and cigarette consumption to match the tumor patients studied.

Major Findings:

The mean serum CEA levels in the smokers was 2.8 ng/ml and the upper limit of normal was set at 2 standard deviations above the mean. This defined the upper limit of normal for nonsmokers was 5.0 ng/ml and for smokers it was 7.0 ng/ml. Five percent of nonsmokers and 13% of smokers had CEA levels that exceeded these limits. Twenty-six (36%) of tumor-bearing patients had CEA levels of 5.0 ng/ml or greater. All gave a history of heavy cigarette consumption. Using the upper discriminate level of 7.0 ng/ml, 30 (17%) had elevated CEA levels, which exceeded the number of elevations in smokers and nonsmokers ($p < .001$). In addition, the number of markedly elevated CEA levels (> 15 ng/ml) was greater in the cancer patients than in smokers or nonsmokers. The levels in the tumor-bearing patients also correlated with tumor burden as determined by clinical staging, with the highest levels occurring in the patients with the most advanced stage of tumor. In patients treated by definitive surgery, there was no correlation between initial CEA level and subsequent clinical course. In 10 patients with elevated preoperative levels, the levels were below the normal range at 1 month postoperatively. However, among 14 patients who developed recurrence after operation, in 10 the levels were normal at the time of recurrence. In patients with elevated CEA levels who received radiation therapy, the levels declined progressively during treatment, paralleling tumor regression. In patients with normal levels prior to irradiation, the levels did not rise during radiation therapy. Selectively fewer abnormal CEA levels occurred in patients tumor-free less than 2 years than in patients tumor-free greater than 2 years, and in both groups the levels were less than in the tumor-bearing patients. In both groups, the levels were similar to that of the smokers, but were higher than in nonsmokers.

Significance to Cancer Research and the Program of the Institute:

The ideal tumor marker would provide an index of prognosis by indicating the presence of occult metastases in patients with clinically localized tumors. Such correlations have been demonstrated in patients with carcinomas of the colon, lung, and breast. However, the data indicate that CEA is not useful for this purpose in patients with carcinomas of the head and neck region. Relevant to this consideration is the ease with which recurrences can be detected in patients with carcinomas of the head and neck region compared to the relative difficulty in detecting early residual disease in malignancies involving the visceral organs. However, the fall in CEA levels after surgical resection, the lack of elevation in CEA levels during radiation therapy, and the decline in CEA levels as the tumors responded to radiotherapy all indicate that CEA may be useful when combined with other serum markers of tumor presence in determining the response of tumor to treatment and in the earlier detection of metastases.

Proposed Course:

Studies in progress evaluate serum carbohydrate and glycoprotein levels in these patients. Comparisons will be made with CEA and *in vitro* correlates of immune reactivity in these patients and in patients with other malignancies in an attempt to derive a group of serum markers which correlate with tumor burden and show alterations prior to the development of clinically manifest residual disease. This study demonstrates that the panel of markers derived will vary with tumor histology and anatomical location.

Project Description:

Part III. Serum Glycoprotein Carbohydrate Levels in Patients with Solid Malignancies

Objectives:

Changes in serum glycoproteins have been documented in many pathologic processes including malignancies. Alteration in the serum concentrations of glycoproteins thus may be of potential value in the determination of tumor burden. The carbohydrate moieties of the glycoproteins, neutral hexose, fucose, sialic acids, hexosamines, also have been shown to be elevated in patients with malignancies. It is relevant here that carcinoembryonic antigen (CEA) is a glycoprotein rich in these carbohydrates and has been shown to parallel tumor burden after treatment in patients with elevated levels of CEA prior to therapy. Additional studies indicate that glycoproteins may suppress immune reactivity. The purpose of this study was to correlate glycoprotein-associated monosaccharides and a group of 17 glycoproteins with tumor burden, lymphocyte reactivity *in vitro*, and serum CEA levels.

Methods Employed:

The patients studied had a variety of solid malignancies clinically staged as localized, metastatic to regional lymph nodes, and disseminated. All blood specimens were obtained prior to treatment. Controls consisted of healthy adults who did not smoke or drink and an additional group of heavy smokers. The control groups were matched for mean age and for sex with the cancer patients. Serum protein-bound carbohydrates and serum glycoprotein levels were determined in the laboratories of the Armed Forces Radiobiology Research Institute under a contractual arrangement. Serum CEA levels were determined by the Hansen Z gel assay. Lymphocyte reactivity to phytohemagglutinin (PHA) was determined in microculture.

Major Findings:

The mean levels of the protein-bound carbohydrates in tumor-bearing patients were significantly higher than in both nonsmoking and smoking normal controls. When the patients were divided into those with local, regional, and disseminated tumors, the mean levels of each carbohydrate increased progressively with increasing tumor burden. Furthermore, the mean levels between each clinical tumor stage also differed. In a group of patients tumor-free for more than 3 years, the serum-bound carbohydrate levels were lower than that of patients with localized tumors but higher than the controls. The mean serum CEA levels in all subpopulations of patients studied did not differ from normals, was elevated compared to nonsmokers, but did not differ from that of smokers. There was no correlation between serum protein-bound carbohydrate levels and CEA levels. The most consistently elevated glycoproteins were the alpha-1-acid glycoprotein, alpha-1 antitrypsin, haptoglobin, and ceruloplasmin. These proteins showed a relationship to tumor burden similar to the protein-bound carbohydrates. There was an inverse relationship between serum-bound carbohydrate levels and lymphocyte reactivity. The best correlation was with sialic acid; however, the correlation was not significant. Analysis of the data suggested that study of larger numbers would be required to demonstrate the relationship at a significant level, because of the variability of the lymphocyte reactivity assay. Among the glycoproteins, the alpha-2-HS-glycoprotein correlated significantly (inversely) with lymphocyte reactivity ($p < .01$).

Significance to Cancer Research and the Program of the Institute:

In this study, each of the protein-bound carbohydrates was shown to be related to tumor burden in patients with solid malignancies and was shown to be a better correlate of tumor burden than CEA. Glycoproteins having a high content of sialic acid showed similar correlations. These observations, combined with the inverse correlations with lymphocyte reactivity in vitro indicate that further studies of larger groups of solid malignancies of well defined histologic types (e.g., squamous carcinomas, adenocarcinomas, melanomas, and sarcomas) are warranted because of this preliminary data indicative of correlation with tumor burden. Demonstration of a correlation in individual patients studied sequentially may allow the earlier detection of residual disease and thus the early institution of adjuvant therapy. Conversely, in patients receiving adjuvant therapy on a long-term basis, these assays may assist in determining the effect on subclinical tumor of the adjuvant.

Proposed Course:

Unfortunately, the contractual arrangements with the Armed Forces Radiobiology Research Institute for analysis of the protein-bound carbohydrates and glycoproteins was limited to 79 specimens. The data indicate that additional specimens will be required to define the relationship between these moieties and tumor burden. At the present time the contract is terminated. Should the contract be reinstituted, additional studies as outlined above will be undertaken to determine the adjunctive value of those markers showing a high correlation with tumor burden and immune reactivity (sialic acid, as alpha-1-acid glycoprotein and alpha-2-HS-glycoprotein). The primary goal of the studies would be to determine their value in monitoring the host-tumor relationship in patients receiving adjuvant chemotherapy and immunotherapy after definitive local treatment of solid malignancies.

Publications:

Silverman, N.A., Alexander, J.C., Jr., and Chretien, P.B.: CEA levels in head and neck cancer. Cancer 37:2204-2211, 1976.

Alexander, J.C., Jr., Silverman, N.A., and Chretien, P.B.: Effect of age and cigarette smoking on carcinoembryonic antigen levels. JAMA 235:1975-1979, 1976.

Smith, H.G., Horowitz, N., Silverman, N.A., Henson, D.E., and Chretien, P.B.: Humoral immunity to herpes simplex viral-induced antigens in smokers. Cancer 38:1155-1162, 1976.

Smith, H.G., Chretien, P.B., Henson, D.E., Silverman, N.A., and Alexander, J.C., Jr.: Viral-specific humoral immunity to herpes simplex-induced antigens in patients with squamous carcinoma of the head and neck. Amer. J. Surg. 132:541-548, 1976.

Bradley, W.P., Evans, A.S., Weiss, J.F., Alexander, J.C., Jr., Silverman, N.A., and Chretien, P.B.: Serum glycoprotein carbohydrates and solid malignancies. Proc. 3rd Int. Symp. on Det. & Prev. of Cancer, New York, 1976.

Crnic, D.M., Weiss, J.F., and Chretien, P.B.: Correlations among serum glycoproteins, *in vitro* lymphocyte reactivity to phytohemagglutinin, T cell levels and tumor burden in patients with head and neck squamous carcinomas. *Proc. Amer. Assoc. Clin. Res.* 18:155, 1977.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 03811-03 SURG

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

The Immunotherapy of Animal and Human Sarcomas

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	S. A. Rosenberg	Chief of Surgery, NCI	SURG NCI
Other:	G. A. Parker	Clinical Associate	SURG NCI
	W. P. Thorpe	Clinical Associate	SURG NCI
	E. H. Austin	Clinical Associate	SURG NCI
	N. Wolmark	Expert	SURG NCI
	S. Schwarz	Bio. Lab. Tech.	SURG NCI
	H. Anding	Bio. Lab. Tech.	SURG NCI
	H. Wexler	Biologist	SURG NCI

COOPERATING UNITS (if any)

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NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
6.5	3.5	3.0

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Detailed studies of tumor-host immune interactions in animals and humans with sarcomas are being performed in an attempt to develop new immunodiagnostic and immunotherapeutic techniques for diagnosis and treatment of these tumors. Immune response to murine sarcomas has been extensively evaluated and both tumor-specific and fetal antigens have been identified. Fetal antigens have been identified on human osteogenic sarcomas and these antigens are now being characterized. Attempts are being made to develop adoptive immunotherapeutic techniques utilizing transfer of cells from xenogeneic species to humans with cancer.

PHS-6040
(Rev. 10-76)

Project Description:

Part I. Antigens on Murine Sarcomas

Part II. Immune Response to Tumors in Patients with Soft Tissue and Bony Sarcomas

Part III. Studies on the Adoptive Immunotherapy of Cancer

Project Description:

Part I. Antigens on Murine Sarcomas

Objectives:

To study the humoral and cellular responses of the host to growing sarcomas. To study and characterize tumor-associated antigens on murine sarcomas. To develop methods of immune manipulation to affect the growth of sarcomas in animal and human hosts.

Methods Employed:

Amputation-challenge experiments have been performed to identify a series of immunogenic sarcomas in mice and rats. A variety of serologic assays have been developed to study the antibody response to tumor-associated antigens on these sarcomas. The major humoral assays that have been evaluated are the C1 fixation transfer test, complement mediated cytotoxicity as measured by microcytotoxicity in microtiter plates and Chromium-51 release, and the radioactive isotope hemadsorption assay. Antibodies in tumor-bearing and immunized mice have been studied. Absorption with a variety of autochthonous syngeneic and fetal tissues have been employed to identify the biologic nature of tumor-associated antigens.

Major Findings:

Many immunogenic sarcomas in mice and rats have been identified that do not cross react with each other in vivo in amputation-challenge experiments. Two distinct tumor-associated antigens have been identified on murine sarcomas. One antigen is specific to each individual tumor and does not cross react with other tumors. A second antigen has been identified that is cross reactive among all murine sarcomas thus far studied. This antigen is also expressed on murine fetal tissue during the first and second trimesters of gestation. Antibody reactivity against the shared fetal component can be removed, leaving antibodies specific for each individual sarcoma. It appears that the tumor-specific antigen and not the shared fetal determinant is responsible for in vivo protection in this murine tumor model.

The fetal antigen present on these murine sarcomas has also been identified on normal cells grown in tissue culture. This expression of oncofetal antigens by normal cells in culture is probably due to dedifferentiation of these cells in tissue culture. Serologic studies of tumor antigens have been extended to animals with primary autochthonous tumors and antibodies similar to those described to those have been found.

Significance to Cancer Research and the Program of the Institute:

Understanding the biologic and immunologic characteristics of murine sarcomas will provide valuable information for developing rational immune manipulations for control of these tumors. This information will be of value in designing immunotherapies for human sarcomas.

Proposed Course:

This study will be continued during the next year.

Project Description:

Part II. Immune Response to Tumors in Patients with Soft Tissue and Bony Sarcomas

Objectives:

To study the humoral and cellular responses of the human host to growing sarcomas. To evaluate the effects of therapy on the immune response to these sarcomas. To develop methods of immune manipulation to improve the treatment of patients with soft tissue and bony sarcomas.

Methods Employed:

Tissue culture techniques have been developed to establish in tissue culture tumor and normal skin from patients in the Surgery Branch with soft tissue and bony sarcomas. Immunologic assays have been developed to monitor the immune response of patients to their own tumors. These assays have largely involved measurements of antibody cytotoxicity as measured by microcytotoxicity tests in microtiter plates and Chromium-51 release assays. A radioactive isotope hemadsorption assay has also been evaluated. Adsorption studies have been performed to identify the nature of the antibody response found in tumor-bearing patients to their autochthonous tumors.

Major Findings:

Both tumor and normal tissue culture lines have been started from 22 patients with osteogenic sarcoma. These cultures have been used to study the immunologic response of these patients to their autochthonous tumors. Antibodies have been identified in tumor-bearing patients that react with their own tumors as well as their normal skin and culture. Absorption studies have revealed that the reactivity of patients' sera with both normal and sarcoma tissue culture lines is due largely to the expression of fetal antigens by these lines. Fetal absorption with human fetal tissue is capable of removing this activity. Sixty-six percent of normal humans contain natural antibodies to these fetal antigens. Quantitative absorption studies of sarcoma patients' sera have revealed the presence of a second antigen, also present on the fetus that appears to be unique to the sarcoma cultures. Presence of antibody to this sarcoma associated antigen is being studied in both normal and sarcoma-bearing patients. Absorption studies have also revealed that a variety of fresh tumors also express the same oncofetal antigen expressed on normal human cells in culture.

Serial studies of immune competence have been performed in patients with soft tissue and bony sarcomas and have documented the immunosuppression resulting from chemotherapy and radiotherapy.

Significance to Cancer Research and the Program of the Institute:

Soft tissue and bony sarcomas in humans have an overall 5-year survival of about 40%. Our studies in the laboratory are aimed at developing immunotherapeutic methods for improving the treatment of patients with these soft tissue and bony sarcomas.

Proposed Course:

This study will be continued during the next year.

Project Description:

Part III. Studies on the Adoptive Immunotherapy of Cancer

Objectives:

To develop a new technique for the immunotherapy of cancer based on the transfer of sensitized xenogeneic cells to tumor-bearing humans.

Methods Employed:

Attempts are being made to develop the best methods for immunizing minipigs to tumor-specific antigens of both rat and animal tumors. The presence of cytotoxic cells is being evaluated by assays involving chromium release as well as the release of IUDR labelled cells from plastic microtiter plates.

Major Findings:

This project has begun in the past several months. Varieties of techniques for immunizing pig spleen and mesenteric lymph nodes to allogeneic antigens are being explored. Preliminary results indicate that intraperitoneal and intramuscular injection can sensitize xenogeneic cells.

Significance to Cancer Research and the Program of the Institute:

Adoptive immunotherapy of cancer may represent a new approach to the treatment of malignant disease that will be of value in treating humans with cancer.

Proposed Course:

This study will be continued during the next year.

Publications:

Parker, G.A., Hyatt, C., and Rosenberg, S.A.: Normal adult murine cells in tissue culture express fetal antigens. Transplantation 23:161-163, 1977.

In Press:

Parker, G.A., and Rosenberg, S.A.: Serologic identification of multiple tumor-associated antigens on murine sarcomas. J. Nat. Cancer Inst.

Parker, G.A., and Rosenberg, S.A.: Cross reacting antigens in chemically induced sarcomas are fetal determinants. J. Immunol.

Thorpe, W.P., Parker, G.A., and Rosenberg, S.A.: Expression of fetal antigens by normal human skin cells grown in tissue culture. J. Immunol.

Rosenberg, S.A.: Lysis of human normal and sarcoma cells in tissue culture by normal human serum: Implications for experiments in human tumor immunology. J. Nat. Cancer Inst.

Rosenberg, S.A., Schwarz, S., Anding, H., Hyatt, C., and Williams, G.M.: Comparison of multiple assays for detecting human antibodies directed against surface antigens on normal and malignant human tissue culture cells. Clin. Exp. Immunol.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 03812-03 SURG

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Studies with a Chemically-Induced Rat Colon Cancer Model

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	A. R. Baker	Senior Investigator	SURG	NCI
Other:	D. F. Devereux	Clinical Associate	SURG	NCI
	E. W. Chu	Head, Cytopathology Section	LP	NCI
	J. Costa	Expert, Viral Oncology & Molecular Pathology	LP	NCI

COOPERATING UNITS (if any)

LAB/BRANCH
Surgery Branch

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INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS: 2.0	PROFESSIONAL: 1.0	OTHER: 1.0

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

DMH-induced rat colon cancer is being explored as a model of human colorectal cancer. Studies are designed to assess the importance of gut transit time as a variable in the carcinogenesis of these chemically-induced tumors. Efforts are being made to determine the immunogenicity of the tumor and to examine tumor-host manipulations which lead to enhanced resistance to this neoplasm.

Project Description:

Objectives:

To explore the utility of a chemically-induced colon tumor in a rat system as a model of human colorectal cancer.

Methods Employed:

Two different DMH-induced colon tumors are carried by subcutaneous passage in syngeneic Buffalo strain rats. Lack of availability of these rats over the past year has seriously hampered progress of studies on tumor immunogenicity.

Gut transit time has been quantitated in the rat by measuring the rate of fecal elimination of radioactive sodium dichromate instilled by orogastric tube.

Major Findings:

(1) Rats maintained on Vivonex alone (low residue) have slower gut transit time (57.9 ± 2.6 hours) than chow fed controls (27.4 ± 2.6 hours).
(2) Restoration of dietary bulk by feeding pre-mixed Vivonex-Methylcellulose returns transit time (26.2 ± 1.8 hours) to that of chow fed controls (27.4 ± 2.6 hours).

Significance to Cancer Research and the Program of the Institute:

The magnitude of the colorectal cancer problem in man makes this animal model system very attractive as a focus for studying pertinent clinical questions.

Proposed Course:

(1) Studies are planned to examine the importance of gut transit time in the carcinogenesis of DMH-induced tumors. Latency period, number of tumors induced, and pattern of tumor distribution will be determined in rats given carcinogen and maintained on either Vivonex, Vivonex-Methylcellulose, or a chow diet.
(2) Further immunologic studies with this tumor will have to await either improved availability of Buffalo rats or the development of new tumors in another syngeneic strain as a spin-off of #1 above.

Publications:

None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 03817-03 SURG

PERIOD COVERED
July 1, 1977 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Cryopreservation of Lymphocytes and Parathyroid Glands

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	H. F. Sears	Senior Investigator	SURG NCI
Other:	S. A. Rosenberg	Chief of Surgery, NCI	SURG NCI
	G. A. Parker	Clinical Associate	SURG NCI

COOPERATING UNITS (if any)

Litton Bionetics

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NCI, NIH, Bethesda, Maryland 20014

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3.0	2.0	1.0

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Lymphocytes from both normal volunteers and cancer patients are cryopreserved and stored for varying lengths of time. These are recovered and used in assays of cellular immune function, and compared to those same assays that are done on fresh cells. These assays are also done in longitudinal fashion on patients undergoing multimodality treatment of cancer. Assays of mitogen stimulation, mixed lymphocyte response, and subpopulation percentages are similar using both fresh and cryopreserved cells. Current methods of cryopreservation may alter the functional response of cells obtained from cancer patients receiving combined modality therapy. This is being evaluated and alternate techniques explored. Parathyroid tissue has been frozen and stored for one to 9 months and reimplanted in parathyroidectomized autologous animals. These endocrine tissues have been able to sustain life, serum calcium levels, and produce parathormone after reimplantation. Serum calcium levels have been evaluated with respect to rate of successful implantation. Pancreatic islet cells are harvested from inbred strains of rats, cryopreserved and stored, and reimplanted into streptozotocin-induced diabetic syngeneic rats. The function of these cryopreserved islets has been successful only in an occasional experiment to date.

PHS-6040
(Rev. 10-76)

Project Description:

Part I. Cryopreservation of Lymphocytes

Part II. Cryopreservation of Endocrine Tissue

Part III. Cryopreservation of Parathyroid Tissue

Project Description:

Part I. Cryopreservation of Lymphocytes

Objectives:

The objective of this study is to cryopreserve immunocompetent cells for subsequent immunologic study of cancer patients undergoing combined modality therapy.

Methods Employed:

Human lymphocytes are separated from normal volunteers' blood as well as the blood of cancer patients who are being treated by the Surgery Branch with combined modality techniques for melanoma, osteogenic sarcoma and soft tissue sarcomas. Lymphocytes are separated and used in assays immediately or cryopreserved.

Major Findings:

After optimization of cryopreservation techniques, lymphocytes from normal volunteers were evaluated. Nonspecific mitogen stimulation, one and two way mixed lymphocyte culture assays, and estimations of mononuclear cell populations were carried out on these tissues. Normal volunteer cells performed adequately in these assays, and had similar populations of recovered lymphocytes with some diminution of the number of nonspecific esterase-containing monocytes. Assay variability using cryopreserved cells was as good as or better than assays done individually on fresh tissue. This work also demonstrated that both Mitomycin C treated lymphocytes and irradiated lymphocytes could be cryopreserved and act as reliable stimulators in these assays. The results obtained from the longitudinal study of cancer patients' immune status have failed to reveal predictive changes prior to the recurrence of gross clinical disease. Of interest, a profound depression in the assays used was noted in those patients having clinical BCG-osis after intralesional injection of their melanomas. Also it has been noted that the cells harvested from patients undergoing multimodality therapy that has included irradiation have mononuclear cells that are more difficult to harvest as a clean preparation, and those cells that are harvested do not react as did the fresh preparation with the same statistical frequency as those patients who have not had radiation therapy or were normal volunteers.

Significance to Cancer Research and the Program of the Institute:

Adequate cryopreservation techniques for immunocompetent cells will provide the investigator with stored material for assays of cellular immunity and tumor immunobiology.

Proposed Course:

We will continue to investigate the use of long-term storage of cryopreserved cells and their ability to function in assays of cellular immunity. Patients undergoing protocol directed chemoimmunotherapy will be studied using simultaneous assay of these frozen storage cells.

Project Description:

Part II. Cryopreservation of Endocrine Tissue

Objectives:

We wish to preserve human endocrine tissue for transplantation as an aid to the therapy of cancer of the thyroid, hyperparathyroidism, and diabetes.

Methods Employed:

Two groups of mongrel dogs have had their parathyroid glands removed, cryopreserved and stored for varying lengths of time. After storage, these glands are reimplanted in the autologous host. In the preliminary study after function was demonstrated by rising serum calcium levels, the graft bed was explored, serum parathormone levels were determined in the venous effluent blood as compared to systemic levels. The graft beds were resected for histologic examination and the dogs were observed until they became tetanic or died. In the second group of animals, matched pairs were either treated with a vitamin D analog pre- and postoperatively to maintain a high serum calcium or given placebo. This was done to evaluate whether a low serum calcium or endocrine stimulus was necessary for implantation of endocrine glandular tissue. These dogs were then treated as the initial pilot group.

Major Findings:

Of the preliminary group of dogs, 14 were suitable for evaluation. Cryopreserved transplanted glands in 10 of these maintained normal serum calcium levels and had viable tissue demonstrated by histologic evaluation and furthermore parathormone was elaborated by the graft bed. Preliminary results of the second study would imply that high levels of serum calcium during the implantation or grafting period causes no loss of ability for the autografted tissue to implant, function, and grow locally.

Significance to Cancer Research and the Program of the Institute:

These studies demonstrate that parathyroid cryopreservation and autotransplantation can successfully restore parathyroid function in a large animal model. Clinically this technique will be useful in the management of parathyroid hyperplasia, total thyroidectomy and re-operation for hyperthyroidism.

Proposed Course:

Further investigation will be carried out to develop the best techniques for cryopreservation of both lymphoid and endocrine cells to insure not only viability, but function, providing an accurate assessment of the material in its fresh state.

Project Description:

Part III. Cryopreservation of Parathyroid Tissue

Objectives:

Preservation of human parathyroid tissue would allow (a) a tissue bank for subsequent autotransplantation, and (b) an ongoing store of human tissue to work with in various experimental studies.

Methods Employed:

Human parathyroid tissue has been frozen from all patients who have had abnormal parathyroid tissue removed in the last year. Subsequently the viability of the tissue is assessed at various periods after cryopreservation by the ability of the cell suspension obtained from the cryopreserved parathyroid tissue to respond with appropriate parathormone release to changing levels of ambient calcium.

Major Findings:

We have shown that by this technique the cryopreserved parathyroid tissue is functional. This has been further documented by the autotransplantation of cryopreserved parathyroid tissue to 4 patients.

Significance to Cancer Research and the Program of the Institute:

This study demonstrates in two different manners the fact that cryopreserved parathyroid tissue will function both in vitro and in vivo. Most importantly this allows us to remove parathyroid tissue from those patients who had multiple operative procedures for their disease previously and in whom it is impossible to decide whether they have parathyroid tissue remaining. By this method we can now, at a subsequent date when the patient is thought to be proven to be aparathyroid, transplant the patient with his own tissue with a good chance of long-term success.

Proposed Course:

This study will continue as we develop better methods of both cryopreservation and ongoing efforts of assessment of parathyroid function, both in vitro and in vivo.

Publications:

Sears, H.F., and Rosenberg, S.A.: Advantages of using cryopreserved lymphocytes for the sequential evaluation of human immune competence. I. Mitogen stimulation. J. Nat. Cancer Inst. 58:183, 1977.

Sears, H.F., and Rosenberg, S.A.: Increased reliability of cellular immune assays in humans by the use of cryopreserved lymphocytes. Surg. Forum 17:162, 1977.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 03818-03 SURG

PERIOD COVERED

July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Cellular Immunity in the Tumor-Bearing Host

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	M. H. Cohen	Senior Investigator	SURG NCI
Other:	R. B. Herberman	Chief, Lab. of Immunodiagnosis	LID NCI
	J. L. Weese	Clinical Associate	LID NCI

COOPERATING UNITS (if any)

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Surgery Branch

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NCI, NIH, Bethesda, Maryland 20014

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1.5	1.0	0.5

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The influence of isoniazid (INH) therapy on the effectiveness of intralesional BCG was studied in the guinea pig-hepatoma model of Rapp and Zbar, in which the intratumor injection of BCG is highly curative. INH, in the schedules and dosages that we used, did not interfere with the antitumor effect of intralesional BCG, although early INH therapy appeared to prolong the interval between intralesional BCG injection and clinical tumor cure. Six week old Balb/c mice were immunized with an allogeneic tumor and serially tested in a microcytotoxicity assay and in a Winn assay, in which immune serum, spleen cells, or peritoneal exudate cells were mixed with melanoma cells and injected into normal mice. The Winn assay appeared to give the results that were most consistent and interpretable. Vitamin A inhibits the growth and development of S-91 melanoma in Balb/c mice. The required dose for this effect is 3500 u/day for 5 days. Vitamin A treatment can be given after tumor inoculation. Vitamin A was effective in transiently slowing the appearance of tumor nodules in one experiment with a histocompatible melanoma. In one of two experiments with syngeneic MCA-sarcoma in Balb/c mice vitamin A delayed and slightly decreased tumor deaths.

PHS-6040
(Rev. 10-76)

Project Description:

Part I.	Effect of Isoniazid on the Development of BCG-Induced Immunity in the Tumor-Bearing Guinea Pig
Part II.	A Comparison of Immune Assays in Mice Immunized with S-91 Melanoma
Part III.	The Effect of Retinoid Compounds on Murine Tumors

Project Description:

Part I. Effect of Isoniazid on the Development of BCG-Induced Immunity in the Tumor-Bearing Guinea Pig

Objectives:

The purpose of this study was to determine whether or not INH would abrogate the therapeutic effect of intralesional BCG injected into metastasizing tumor nodules in the strain 2 guinea pig.

Methods Employed:

Seventy-five strain 2 male Sewall-Wright guinea pigs, with a mean weight of 492 gm, were each inoculated intradermally with 10^6 viable hepatoma ascites cells, a dose which kills 100% of untreated animals. Six days after tumor inoculation, 0.1 ml of Tice BCG ($5\pm3\times10^7$ viable organisms) was injected intralesionally into each palpable 1 cm tumor nodule. This regularly results in cure of over 80% of animals. In the INH treated animals, 0.5 mg INH per 100 gm body weight was given intramuscularly for a maximum of 3 months. This daily dosage of INH corresponds to the usual human dosage recommended for the treatment of tuberculosis.

Major Findings:

INH alone did not affect tumor growth. When INH therapy was begun either 6 days before BCG inoculation, or simultaneously with BCG, or 3, 7, 14, 21, or 28 days after intralesional BCG (4-10 animals per group), 50-100% of the animals survived, indicating normal effectiveness of a single BCG intralesional injection despite INH therapy (no significant difference between groups and controls). Early INH therapy did seem to prolong the time before which the animals were clinically cured of palpable tumors: 7/8 (88%) of the animals receiving BCG without INH therapy were tumor-free after one month while only 11/28 (39%) of the animals receiving INH (initiated 6 days before, simultaneously with, or 3 days post-BCG) were tumor-free by the same time ($p = 0.05$).

Significance to Cancer Research and the Program of the Institute:

Patients with melanoma being treated with intralesional BCG occasionally require INH therapy for complications of BCG dissemination. This raises the question of whether or not INH would also abrogate any therapeutic effect of BCG. The guinea pig-hepatoma model of Rapp and Zbar is the main animal model for demonstrating the ability of intralesional BCG to obliterate microscopic lymph node tumor metastases. In this study, INH did not prevent tumor cure mediated by intralesional BCG, in the dosages and schedules used.

Proposed Course:

Higher doses of INH and larger numbers of animals are currently being studied to further evaluate the questions proposed.

Project Description:

Part II. A Comparison of Immune Assays in Mice Immunized with S-91 Melanoma

Objectives:

The purpose of this study was to serially test immune reactivity in Balb/c mice immunized with S-91 melanoma and tested simultaneously by a variety of techniques.

Methods Employed:

Balb/c mice were immunized with 10^5 irradiated S-91 cells and Freund's adjuvant, followed by an intraperitoneal boost with $4x10^5$ S-91 irradiated cells without Freund's adjuvant, followed by subsequent challenge with viable tumor. Cytotoxicity assays were performed serially by plating 10^3 viable cells per well in a microtest plate, labelling with ^{125}I deoxyuridine, and adding pooled lymphocytes or pooled serum or pooled peritoneal exudate cells.

In other mice a Winn assay was performed by mixing 10^5 tumor cells with 10^7 splenic lymphocytes and/or 0.1 cc of pooled serum. The mixture was incubated 30 minutes at 37° and injected subcutaneously between the scapulae of normal Balb/c mice who were observed for tumor take and survival. Concomitant immunized Balb/c mice were serially tested to confirm their solid immunity to a 10^5 challenge inoculum.

Major Findings:

On serial testing on the immunized mice through the period of immunization, boosting, and tumor challenge, the Winn assay results consistently showed that the immune splenic lymphocytes and the immune peritoneal exudate cells were capable of killing admixed melanoma cells. The concurrent tests in the microcytotoxicity assay were not as easy to correlate with the *in vivo* tumor immunity, because of the cytotoxicity of normal serum against the S-91 melanoma cells and because of the low cytotoxic efficacy of the immune cells.

Significance to Cancer Research and the Program of the Institute:

This study represents an attempt to correlate serially the in vitro tests and the in vivo findings.

Proposed Course:

We plan to utilize this assay to assess the mechanisms of action of retinoid compounds and to apply the technique to the histocompatible S-91/CDF system and to further studies on the peritoneal exudate cells. These latter cells are thought to be stimulated by glucan, a compound whose antitumor effects we are also studying.

Project Description:

Part III. The Effect of Retinoid Compounds on Murine Tumors

Objectives:

The purpose of these studies was to further our observations in a murine melanoma system by studying the effect of antilymphocyte serum (ALS) on the protection afforded by vitamin A, and to determine the antitumor effect of vitamin A administration at different times in relationship to the tumor challenge. We studied several syngeneic systems as well.

Methods Employed:

To determine the interaction between immune suppression and the vitamin A inhibition of S-91 growth, Balb/c mice were given what had been previously determined to be a threshold (10^4 cells) S-91 dose or a standard (10^5 cells) S-91 challenge. They were also given 0.25 ml of either ALS, normal rabbit serum (NRS) or saline immediately before and 1, 3, and 5 days after tumor challenge. In some experiments a 5 day course of vitamin A was given 6 weeks prior to tumor challenge and in other experiments it was given beginning 10 days following tumor challenge in the S-91 melanoma experiments. In subsequent experiments vitamin A was given for 5 days immediately prior to or immediately following inoculation with a syngeneic MCA tumor. Other syngeneic tumors were studied as will be indicated.

Major Findings:

Mice inoculated with 10^4 S-91 cells and subsequently treated with ALS had a significant increase in tumor incidence compared to mice treated with saline or normal rabbit serum. Vitamin A, when given in addition to ALS, prevented tumor growth and thus reversed the ALS enhancement of growth following the threshold S-91 inoculum. Vitamin A treatment was not able to reverse the ALS enhancement following a standard (10^5 cells) tumor challenge.

Vitamin A had an antitumor effect when begun in the allogeneic S-91 system immediately following tumor inoculation, in contrast to our previous reports in which vitamin A was always given prior to tumor inoculation. However, when vitamin A was given 6 weeks prior to tumor inoculation or 10 days after tumor inoculation, the decrease in tumor incidence in the vitamin A-treated animals was not statistically significant.

When S-91 melanoma was given to histocompatible CDF_1 mice there was, in multiple experiments, no significant difference in survival of the vitamin A-treated mice compared to control mice. In one experiment, however, there was a delay in the appearance of palpable tumors 13 days after tumor inoculation in the vitamin A-treated animals compared to the normal control animals ($p < 0.025$). By 19 days this difference had disappeared.

Balb/c mice inoculated with $2x10^3$ syngeneic MCA cells and treated with vitamin A for 5 days either before or after tumor inoculation experienced a death rate of 72% (28/39). The death rate among all of the control animals treated with saline or polysorbate vehicle was 88% (75/85), a difference which was statistically significant. In an attempt to repeat this experiment, the tumor grew more rapidly in all groups and no differences were observed.

Significance to Cancer Research and the Program of the Institute:

Vitamin A has been shown to increase the effectiveness of BCG immunotherapy, chemotherapy, and radiation therapy in experiments with syngeneic tumors. Recently, vitamin A alone has been shown to increase the survival time (but not the survival rate) of mice inoculated with a syngeneic mammary adenocarcinoma. We have shown that vitamin A improves the survival rate following challenge with an allogeneic murine melanoma. The current experiments indicate that in the Balb/c mouse, ALS inhibits the vitamin A effect, and that the vitamin A can be given after tumor challenge. In one experiment with a syngeneic methylcholanthrene-induced sarcoma, survival was slightly but statistically increased in the vitamin A-treated mice. Taken together, these studies indicate that vitamin A may have a role in enhancing the potency of adjuvant therapy, and possibly may have an adjuvant effect itself.

Proposed Course:

We are currently investigating the mechanism of the vitamin A-related tumor protection, and testing retinoid analogs that may have a higher therapeutic/toxic ratio.

Publications:

Felix, E.L., Loyd, B., and Cohen, M.H.: Inhibition of the growth and development of the transplantable murine melanoma by vitamin A. Science 189:886-888, 1975.

Felix, E.L., Cohen, M.H., and Lloyd, B.C.: Immune and toxic anti-tumor effects of systemic and intralesional vitamin A. J. Surg. Res. 21:307-312, 1976.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 03826-03 SURG

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Studies of Genitourinary Tissue Transformed by Oncogenic Viruses and by Chemical Carcinogens

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	N. Javadpour	Senior Investigator	SURG NCI
Other:	None		

COOPERATING UNITS (if any)

LAB/BRANCH
Surgery Branch

SECTION
Office of the Chief

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.5	1.5	1.0

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The presence or absence of cell surface antigens (A,B,H) on the cell surface of bladder tumors is studied. The major blood group antigens A, B, or O (H) are present on normal bladder epithelium but are not present on some low grade and low stage papillary transitional cell carcinomas of the bladder. In this study we have demonstraged that Stage A bladder tumors that have not recurred in at least 5 years 3 of 5 have retained the cell surface antigens while tumors that have recurred locally or invaded or metastasized had lost these cell surface antigens on the original Stage A tumor. We are attempting to correlate these cell surface antigens with various histopathologic grades and stages of the bladder tumor.

PHS-6040
(Rev. 10-76)

Project Description:

Part I. The Cell Surface Antigen A, B, or O (H) as an Indicator of Malignant Potential in Stage A Bladder Carcinoma

Part II. Localization of HCG and AFP in Germ Cell Tumors of the Testis

Project Description:

Part I. The Cell Surface Antigen A, B, or O (H) as an Indicator of Malignant Potential in Stage A Bladder Carcinoma

Objectives:

The major blood group antigens A, B, or O (H) are present on normal bladder epithelium but are not present on some low grade and low stage papillary transitional cell carcinomas of the bladder. The objective of this study is to attempt to predict the natural history of the initial tumor based on the expression of A, B, or O blood group antigens.

Methods Employed:

Five micron thick histologic sections of bladder tumors were assayed for blood group antigens by hemagglutination techniques.

Major Findings:

Five patients had no recurrences. Three had positive specific red cell adherence (SRCA) of at least 2+, the other 2 patients were negative. Nine patients had recurrences, of which 6 recurred only as Stage A Grade I or II during the follow-up period. All had 0 or 1+ SRCA. Three patients with recurrence with invasion or metastases all had negative SRCA.

Significance to Cancer Research and the Program of the Institute:

The initial results indicate that those Stage A bladder tumors that have not recurred have retained the cell surface antigens. Those tumors that have recurred locally or invaded or metastasized have lost the cell surface antigen on the original Stage A tumor. It appears that cellular dedifferentiation is reflected in the loss of tumor cell surface antigens. This study is designed to predict the propensity of the tumor to recur, invade, or metastasize. This assessment will improve the prognosis and survival of these patients by receiving the appropriate therapy based on the loss of cell surface antigen of the tumor.

Proposed Course:

This study is being continued in order to obtain a larger number of patients with Stage A bladder cancer.

Project Description:

Part II. Localization of HCG and AFP in Germ Cell Tumors of the Testis

Objectives:

To identify the cells within germ cell tumors of the testis which contain the tumor markers HCG and AFP.

Methods Employed:

HCG and AFP are found in elevated levels in the circulation of most patients with germ cell testicular tumors other than seminoma. In monitoring the serum levels, however, it is not uncommon to find patients with only one marker elevated while the other remains normal, or to find both markers elevated but only one return to normal after therapy. These discordant serum levels are felt to reflect production of these markers by different populations of cells with different growth rates and susceptibilities to treatment.

Major Findings:

Twenty-nine primary or metastatic tumor specimens from 20 patients whose serum levels of HCG and AFP had been assayed at the time the tissue was obtained. Deparaffinized sections were reacted with specific anti-HCG and anti-AFP antibody in an indirect immunoperoxidase assay. AFP was demonstrated in mononuclear embryonal cells within embryonal carcinoma and endodermal sinus tumor. HCG was identified within syncytiotrophoblastic giant cells frequently in association with embryonal carcinoma, and rarely with endodermal sinus tumor and seminoma as well as in the syncytiotrophoblastic component of choriocarcinoma. Eighteen of 21 patients (86%) had elevated tumor markers in their serum; serum HCG alone was elevated in 5 (24%), AFP alone in 5 (24%) and both were elevated in 8 (38%). There was tissue localization of HCG in 12 of 13 patients (92%) with elevated serum HCG while AFP was identified in the tumor in 8 of 13 patients (53%) with elevated serum AFP levels. Based on these findings a tentative immunohistologic classification of germ cell tumors utilizing AFP and HCG is proposed. Thus embryonal carcinoma, adult type, is frequently associated with both AFP and HCG, endodermal sinus tumor with AFP and choriocarcinoma with HCG, whereas pure seminoma and teratoma are unlikely to be associated with either marker.

Significance to Cancer Research and the Program of the Institute:

The cells responsible for the production of HCG and for AFP in germ cell tumors of the testis were identified. These two markers localized to entirely different cell types, HCG to syncytiotrophoblastic giant cells and AFP to discrete areas of embryonal carcinoma, particularly the "endodermal sinus tumor" components. These findings provide an explanation for the discordance of serum levels of HCG and AFP frequently observed, and strongly suggest that an elevated serum level of HCG or AFP reflects the presence of the corresponding cell type within the tumor. This information can be used in directing treatment toward the actual tumor cell types present in a particular patient at a particular time.

Proposed Course:

Further tissue specimens will be subjected to the immunoperoxidase assay for HCG and AFP to substantiate the findings reported above. Fresh frozen tissue specimens are being collected and will be assayed both for tissue HCG and AFP concentrations and for specific localization to determine whether routine fixation and embedding alter the reactivity of the tissue antigens.

Publications:

None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 06651-02 SURG

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Studies of Transplantation Tolerance

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	D. H. Sachs	Head, Transpl. Biol. Sec.	I	NCI
	S. A. Rosenberg	Chief of Surgery, NCI	SURG	NCI
Other:	G. S. Leight	Clinical Associate	SURG	NCI
	R. L. Kirkman	Clinical Associate	I	NCI
	G. M. Williams	Senior Surgeon	SURG	NCI

COOPERATING UNITS (if any)

W. Flye, Walter Reed Army Hospital

LAB/BRANCH
Surgery Branch

SECTION
Office of the Chief

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
6.0	4.0	2.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

While many advances in transplantation biology have been made possible by the availability of numerous inbred strains of mice, there remains a need for comparable large animal models for organ transplantation. Over the past 3 years we have developed 3 strains of miniature swine, each homozygous for a different allele of the major histocompatibility locus (MHC). To accomplish this in a minimum number of generations, a selective breeding scheme was used. Prior to breeding, the original parents were reciprocally immunized with skin grafts and lymphocyte injections. This produced antisera used in a microcytotoxicity assay with appropriate absorptions to type the offspring of this mating. Selective breeding based on serologic typing of the 62 descendants of this original pairing has led to the development of 3 separate herds, each homozygous at the MHC. Using hyperimmune sera raised by immunizations between members of these 3 homozygous strains, 138 different cytotoxicity combinations were tested on lymphocytes from 2nd to 4th generation pigs. No reactions inconsistent with serologic specificities predicted genetically were obtained, confirming the segregation of serologically defined alleles. Mixed lymphocyte cultures likewise confirmed the serologic genotyping: between 68 pairs of animals tested, the mean two-way stimulation index was 1.00±0.07 for serologically identical animals vs. 9.59±2.94 for animals with one or two haplotype differences.

Project Description:

Objectives:

To develop several strains of miniature swine, each homozygous for a different set of histocompatibility antigens at the major histocompatibility locus (MHC). After documentation of homozygosity, the animals will be used for *in vivo* experiments in organ and tissue transplantation, as a source of large numbers of cells from which cell surface antigens can be isolated and characterized chemically, and in studies of tolerance to antigens administered to fetal pigs *in vitro*.

Methods Employed:

Unlike classical inbreeding schemes which require about 20 generations to produce homozygosity, the approach being used with these miniature swine involves selective breeding on the basis of histocompatibility typing of parents and offspring. Prior to breeding, the original parents were reciprocally immunized with skin grafts and lymphocyte injections. This produced antisera used in a microcytotoxicity assay with appropriate absorptions to type the offspring of this mating. Selective breeding based on serologic typing of the 62 descendants of this original pairing has led to the development of three separate herds, each homozygous at the major histocompatibility locus.

Major Findings:

Using hyperimmune sera raised by immunizations between members of these three homozygous strains, 138 different cytotoxicity combinations were tested on lymphocytes from second to fourth generation pigs. No reactions inconsistent with serologic specificities predicted genetically were obtained, confirming the segregation of serologically defined alleles. Mixed lymphocyte cultures likewise confirmed the serologic genotyping: between 68 pairs of animals tested, the mean two-way stimulation index was 1.00 ± 0.07 for serologically identical animals versus 9.59 ± 2.94 for animals with one or two haplotype differences. Skin graft survivals between serologically identical animals was 11.8 ± 0.88 days versus 7.00 ± 0.36 days for animals with one or two haplotype differences.

Significance to Cancer Research and the Program of the Institute:

One of the major problems in the study of cell surface antigens and organ transplantation in human beings is the lack of control of genetic constitution. The use of inbred mice avoids this problem but because of marked size differences, comparison and application of findings to the human situation is difficult. Additionally, it is extremely difficult to obtain sufficient cells and tissues from rodents to permit quantitative chemical characterization of relevant cell surface antigens. For these reasons it is desirable to have animals of size comparable to human beings whose genetic constitution with respect to histocompatibility could be controlled. Furthermore, studies of transplantation tolerance in experimental systems will be of value in the development of immunotherapeutic approaches to cancer involving the adoptive transfer of immune sensitized cells.

Proposed Course:

The breeding plan will be continued to produce larger herds in each line and some hybrid lines will be established. Transplantation of organ allografts will be performed in order to characterize the immune response to transplantation and the mechanism of enhancement. Studies of the I_a antigen system and the chemical characterization of histocompatibility antigens will be pursued. The possible use of sensitized pig cells in the therapy of human malignancy will also be explored. Extensive efforts are being made to develop the techniques for transplantation of kidneys and livers in pigs and humans. Immunologic analysis of animals completing successful liver and kidney transplantation will be performed.

Publications:

None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 06652-01 SURG

PERIOD COVERED

July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Decreasing Strengths of Cell-Mediated Immune Responses Generated Following Repeated Alloantigenic Stimulation in Inbred Strains of Mice

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	P. H. Sugarbaker	Senior Investigator	SURG NCI
Other:	A. E. Chang	Clinical Associate	SURG NCI
	W. Matthews, Jr.	Research Associate	SURG NCI

COOPERATING UNITS (if any)

H. J. Winn, Assoc. Prof. of Microbiology, Harvard Medical School
Department of Surgery, Massachusetts General Hospital

LAB/BRANCH

Surgery Branch

SECTION

Office of the Chief

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.8	1.8	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Although cell-mediated immune responses to a variety of alloantigenic stimuli have been studied thoroughly in the past, responses that occur after prior alloantigenic stimulation have not been previously thoroughly investigated. Our studies involve an assessment of cell-mediated immune responses after the host has responded to a variety of alloantigenic stimulation to the same and other antigens. We find a marked specific decrease in the generation of cytotoxic cells after previous immunizations. The diminished response is specific for the immunizing alloantigen. Passively transferred high titered alloantisera causes some decrease in cytotoxic cell generation but thus far no decrease occurs following passively transferred cells. Present efforts involve further definition of humoral and cellular mechanisms of the depressed cytotoxicity generated in previously immunized animals. The significance of this work comes in attempts to generate large numbers of specifically programmed cytotoxic cells for adoptive transfer of immunity to tumors.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives:

A profound decrease in cytotoxic responses after repeated prior alloantigenic stimulation has been defined. Our efforts are now focused on the mechanisms whereby this diminished generation of cytotoxic cells occurs. Further definitions of the immunogenetic restrictions, the requirements of the immunizing antigen and means whereby this suppression can be reversed are currently under study.

Methods Employed:

We have used inbred strains of mice of limited alloantigenic diversity as donors and recipients of transplanted tumors or skin grafts. In this study, alloantigens on tumor or tissue grafts have been used as a simplified model for studying tumor immune phenomenon. Repeated immunizations with lymphocytes from a particular strain of mouse followed by skin grafting using the same or different alloantigenic disparity to generate T cell cytotoxic responses constitutes the basic model. Other stimuli for T cell cytotoxicity include solid or ascitic tumors. Serial recovery of cytotoxic lymph node cells, spleen cells, or peripheral blood lymphocytes from grafted mice is accomplished and the cytotoxicity of these cells assayed using ^{51}Cr labelled tumor target cells in vitro. Other in vivo assessments of transplantation immunity involve tumor challenge with both solid and ascitic tumors, and skin grafting.

Major Findings:

The marked decrease in cell-mediated immune responses that occurs after previous alloantigenic stimulation has been documented in a variety of mouse systems. Previously immunized mice show less than 20% of the cytotoxicity in local lymph nodes, spleen or mesenteric nodes as do those mice who are responding to the alloantigen for the first time. This phenomenon is seen in antibody-free systems so is not entirely dependent upon humoral mechanisms. However, the passive transfer of alloimmune antisera does effect a partial decrease in cell-mediated responses. We do find that at the site of tumor rejection, cytotoxic cell populations in hyperimmune and primary immune animals are very nearly the same. It seems that with prior immunization, a population of effector cells is generated that produces a localized cytotoxic response without generation of effector cells in peripheral lymph nodes or spleen.

Significance to Cancer Research and the Program of the Institute:

Much of the immunologic effort of the Surgery Branch is devoted to effecting a successful adoptive transfer of cytotoxic cells programmed to kill tumors. The generation of large quantities of these cells would be required for such an experiment to be successful. This study is in essence an investigation of methods required to generate such cells for immunotherapy. Current data strongly suggest that if a host is going to generate large numbers of cytotoxic cells, it does it most effectively as a primary immune response and does so very ineffectively as a secondary or hyperimmune response.

Proposed Course:

We plan to continue investigating the mechanism of this important immunologic phenomenon. Current studies are now under way to investigate the role early destruction of antigen in the immunized host plays in producing this decreased cytotoxicity. Further investigation of the immunogenetic restrictions of this phenomenon are under way. Methods to counteract this decreased cytotoxicity or to amplify it are being investigated. The passive transfer of diminished response with antisera or with cells is also to be investigated. More direct investigations using the Winn assay will study the cytotoxic potential of hyperimmune and primary immune mice.

Publications:

None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 06653-01 SURG

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Metabolic Studies in Tumor-Bearing Patients and Animals

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	M. F. Brennan	Head, Sec. of Surg. Metab.	SURG NCI
Other:	J. T. Goodgame	Clinical Associate	SURG NCI
	S. F. Lowry	Clinical Associate	SURG NCI
	J. A. Norton	Clinical Associate	SURG NCI
	M. Maher	Clinical Nurse	SURG NCI
	D. White	Computer Analyst	SURG NCI
	C. Gorschboth	Medical Technologist	SURG NCI
	D. Jones	Biologist	SURG NCI
	A. S. Levine	Chief, Ped. Oncol. Br.	PO NCI

COOPERATING UNITS (if any)

LAB/BRANCH
Surgery Branch

SECTION
Surgical Metabolism Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
8.0	5.0	3.0

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The Surgery Branch is vigorously involved in the assessment of the efficacy of total parenteral nutrition as a means of nutritional support of the cancer-bearing host. These studies are directed at conventional nitrogen balance, weight change, and with specific attention to muscle protein breakdown as assessed by 3-methyl histidine excretion in the urine. Studies are currently being initiated to examine specific substrate metabolism in these patients, particularly alanine and glucose. In addition, the first prospective randomized protocol of the use of total parenteral nutrition as an adjunct to aggressive chemo- and radiotherapy has been initiated. Determination of specific DNA activity and conventional parameters of tumor and host growth in animals bearing a rat sarcoma reveal that the tumor is relatively resistant to substrate manipulation, e.g., total starvation, protein depletion, or cortisone administration while the host bears a marked nutritional insult even in the early stages of the cancer-bearing state. An effort has been made to nutritionally deprive human melanoma and osteosarcoma tissue cell lines of essential nutrients along with appropriate fibroblastic controls.

Project Description:

Part I. Metabolic Studies of Tumor-Bearing Patients

Part II. Substrate Manipulation of the Tumor-Bearing Animal: Assessment of Growth Potential

Part III. Nutritional Excess and Deprivation Experiments in Human Cell Lines

Project Description:

Part I. Metabolic Studies of Tumor-Bearing Patients

Objectives:

To develop methods of assessing the efficacy of total parenteral nutrition and to further characterize the metabolic changes that occur in the cancer-bearing host with particular direction at the loss of lean tissue mass and the initiation and control of gluconeogenesis.

Methods Employed:

All the patients are undergoing rigorous therapy for cancer and require nutritional support. They may be members of prospective randomized trials or be hyperalimented as part of the support service of the Surgery Branch. Careful balance studies are obtained along with multiple determination of substrate profiles along with metabolic hormones. Analysis of 3-methyl histidine urine excretion along with other amino acid excretions is identified in the 121MB Beckman autoanalyzer.

Major Findings:

The majority of the studies are preliminary but the initial findings with 3-methyl histidine look most encouraging as a means of assessing lean tissue loss.

Significance to Cancer Research and the Program of the Institute:

One of the major problems of nutritional adjunctive therapy is the burgeoning use of total parenteral nutrition in all aspects of medical and surgical care. Such an entity is extremely expensive and is not without complications. It is imperative that we define the efficacy for the patient and determine the relative contribution of the infused nutrient to either the host or the tumor.

Proposed Course:

This is a long-term ongoing commitment of the Surgery Branch and will require considerable expansion.

Project Description:

Part II. Substrate Manipulation of the Tumor-Bearing Animal: Assessment of Growth Potential

Objectives:

With the advent of total parenteral nutrition in the cancer-bearing human it becomes even more imperative to assess to what extent the infusion of nutrient contributes to host growth as opposed to tumor growth.

Methods Employed:

Using conventional measures of tumor and host growth, i.e., total body weight, tumor-free body weight, tumor and liver size, along with the ability of tumor and host tissues to incorporate tritiated thymidine and total DNA content of the same tissues, an attempt was made to assess these factors.

Major Findings:

Starvation, protein depletion, and cortisone administration all had little influence on tumor growth. However, the host on all occasions had arrested growth. Of particular interest was the observation that in total starvation the tumor had increased specific DNA activity (DPM/μg/DNA), while host tissues fell under the influence of starvation.

Significance to Cancer Research and the Program of the Institute:

Such observations, if able to be extrapolated to man, would extend the justification for total parenteral nutrition with available therapeutic alternatives to attack the tumor. It would emphasize that starvation is indeed bad for the host but has no effect on the tumor and, if anything, encourages tumor growth potential.

Proposed Course:

This study is being expanded to include other methods of substrate manipulation such as diabetic animals, prolonged protein depletion, the effect of total parenteral nutrition in the rat (amino acids alone, amino acids plus glucose, glucose alone) in an effort to better characterize these changes.

Project Description:

Part III. Nutritional Excess and Deprivation Experiments in Human Cell Lines

Objectives:

The objective of this study was to determine whether or not specific amino acids required for metabolism within the melanoma cell (namely phenylalanine and tyrosine) could be removed from the media, maintain growth of the cell line, but diminish its activity, with the long-term projected idea that such manipulations could be carried to the *in vivo* human system because of the ready availability of parenteral nutrition support by vein.

Methods Employed:

Human cell culture lines were plated in control and deficient media (phenylalanine and tyrosine deficient). Growth characteristics were assessed by tritiated leucine incorporation for protein synthesis and tritiated thymidine incorporation for nuclear activity.

Major Findings:

Human malignant melanoma cell lines have been shown to have marked diminution in protein synthesis and tritiated thymidine incorporation under phenylalanine and tyrosine deprivation.

Significance to Cancer Research and the Program of the Institute:

Such projects would allow us to develop more scientifically based nutritional therapy for cancer-bearing hosts.

Proposed Course:

Studies continue with the examination of other cell lines to confirm that this is not a characteristic of a single cell line and, in addition, involve the determination of particular enzyme activity (tyrosine) and the effect of excesses of specific amino acids and their precursors (tyrosine, phenylpyruvic acid).

Publications:

Brennan, M.F., and Goodgame, J.T.: Nutrition in cancer. Host-tumor interaction during varying conditions of substrate availability. In Richards, J.R., and Kinney, J.M. (Eds.): *Nutritional Care of the Critically Ill*. London, Churchill, Livingston, 1977.

Reilly, J.T., Goodgame, J.T., Jones, D.C., and Brennan, M.F.: DNA synthesis in rat sarcoma and liver: The effect of starvation. *J. Surg. Res*. 22:281-286, 1977.

In Press:

Brennan, M.F.: Uncomplicated starvation versus cancer cachexia. *Cancer Res*.

SUMMARY REPORT
ASSOCIATE DIRECTOR FOR BALTIMORE CANCER RESEARCH CENTER
DIVISION OF CANCER TREATMENT
NATIONAL CANCER INSTITUTE
July 1, 1976 - September 30, 1977

The Baltimore Cancer Research Center has completed its second year since transferring to the University of Maryland Hospital. The Clinical Branch including the support services of the Clinical Research Pharmacy Service, Microbiology Section, Infection Research Section, and Cell Component Therapy Unit has produced a number of fruitful projects. During report interval, care has been provided for approximately 12,000 in-patient days and 15,000 out-patient visits. The Laboratory of Clinical Biochemistry and Laboratory of Molecular Biology anticipates completion of renovations to Howard Hall II and moving of their operation within the year. Plans have been completed for development of a new modern and efficient out-patient department, a laminar air flow complex with adjacent intensive care unit and appropriate laboratory support services.

Phase I clinical studies of nitrous oxide have been initiated. Phase II evaluation of levamisole in lung cancer, intrathecal thiotepa in meningeal leukemia, dianhydrogalactitol and piperazinedione in brain tumors, thiotepa, vinblastine, CCNU and piperazinedione in renal cell carcinoma, azotomycin in metastatic sarcoma and adriamycin in mycosis fungoides have all been completed. MER in colon cancer, oat cell carcinoma, previously treated acute leukemia patients and melanoma have been found inactive. New Phase II studies of anguidine in colon cancer have been implemented.

Phase III studies of daunorubicin and cytosine arabinoside in adult acute nonlymphocytic leukemia have demonstrated a 70% complete remission rate. Additional Phase III studies of methyl-CCNU and Ftorafur in colon cancer has been completed and a study of streptozotocin, cyclophosphamide, adriamycin and bleomycin for advanced refractory Hodgkin's disease has indicated a high degree of activity. Cytoxan, adriamycin and VP 16-213 has produced a 40% complete remission rate in patients with oat cell carcinoma of the lung. Combined modality studies of Hodgkin's disease continue to show significant advantage for radiotherapy-chemotherapy in patients with stage IIIA or with earlier stages associated with "E" substage of the lung.

The combination of carbenicillin and gentamycin has been demonstrated as superior for the treatment of febrile agranulocytic cancer patients and research into the causative agents of infection as well as the use of newer aminoglycoside antibiotics has continued. The use of laminar air flow protection of patients with diffuse histiocytic lymphoma permits larger than usual doses of Cytoxan, adriamycin, vincristine and prednisone to be delivered. The value of granulocyte transfusions and methods for increasing normal donor yield as well as a prophylactic granulocyte transfusion study are all in progress. Studies with autologous frozen platelets in acute leukemia patients without compatible donors have shown them to be lifesaving.

Pharmacologic studies of cancer chemotherapeutic drugs, particularly the anthracycline antibiotics have further elucidated the function of microsomal reductive glycosidase. The enzyme has been solubilized and isolated. Phenobarbitol hepatic induction has shown a decrease in the efficacy of adriamycin primarily through increased toxicity. Studies of intracellular carbonyl reductase have demonstrated marked ability to alter pharmacologic activity of a wide spectrum of agents. There are indications that cytoplasmic carbonyl reductase is a family of enzymes, possibly genetically related and with similar characteristics.

Radioimmunoassays for adriamycin and daunorubicin have been developed and compared with more conventional techniques. The anthracyclines also appear to augment electron transfer from NADPH to oxygen with free radical formation thus adding an additional dimension to the mechanism of action of the anthracyclines. Additional studies are being carried out to elucidate this activity as well as the modulation of anthracyclines by hyperthermic conditions.

Cellular regulation through stabilization of messenger RNA in normal and neoplastic tissue has indicated that polyadenylic acid plays a vital role. Specific ribonucleases which are inhibited by polyadenylic acid were isolated and characterized. The removal of polyadenylic acid from messenger RNA and subsequent exposure to ribonuclease produced rapid degradation. Therefore, an important factor in the stabilization of messenger RNA would appear to be the presence of polyadenylic acid. The polyamine spermine was demonstrated to be capable of reversing the inhibition of polyadenylic acid on ribonuclease activity. Thus, multiple modulating effects are being sequentially investigated in normal and neoplastic tissue. The apparent elevation of serum ribonuclease found in patients with multiple myeloma and various leukemias appears more related to renal damage than the basic neoplastic disease.

Immunologic induction of in vitro lymphocyte responsiveness has been measured with laser cytometry capable of defining cellular changes during blastogenesis. The technique has been compared with traditional thymidine incorporation and has demonstrated that exogenous thymidine was not always utilized. The immune response has been shown to be modulated by polynucleotides and beta-blockers. Using B16 melanoma, a single course of chemotherapy followed by surgery and immunotherapy was found to be the most efficacious sequential approach. Patients recently completing radiotherapy or with active recurrent disease, were shown to have markedly impaired cell associated immunity to varicella zoster antigen. Such techniques might be capable of predicting susceptability to viral infection. Serum alkaline ribonuclease activity in normal donors and cancer patients has showed a marked difference in nucleolytic activity. Abnormally elevated activity against one synthetic substraight did not necessarily indicate elevated activity against all.

The Baltimore Cancer Research Center has had another highly productive year of its clinical and laboratory research programs. Participation in onoclogy programs at the two medical schools in Baltimore and hospitals of the community, as well as other Federal and academic institutions throughout the country have developed rich resources and have strengthened collaborative activities at both the basic and applied science level. The continued renovations of the clinical and laboratory areas will provide an unparalleled integrated research unit with all of its components within geographic proximity. The BCRC will continue to be a major clinical and laboratory investigative component of the Division of Cancer Treatment.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 06282-10 BCRC

PERIOD COVERED
July 1, 1976 through September 30, 1977

TITLE OF PROJECT (80 characters or less)

THE BRAIN TUMOR STUDY GROUP

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

Principal Investigators: M.D. Walker, Assoc. Dir., BCRC, CL, NCI
T.A. Strike, Sp. Assist. Prog. Dev., BCRC, CL, NCI

COOPERATING UNITS (if any)

Members of the Brain Tumor Study Group

LAB/BRANCH

SECTION

INSTITUTE AND LOCATION
NCI, NIH, Baltimore, Maryland 21201

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.1	1.1	1.0

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

University affiliated contractors, collectively known as the Brain Tumor Study Group, are cooperating in a continuous effort -- conducting Phase III clinical trials to evaluate chemotherapy, radiation therapy and combinations thereof (multi-modality therapy) following definitive surgery in the treatment of brain tumors (malignant gliomas). New chemotherapeutic agents are also being tested in Phase II clinical trials in order to make the selection of chemotherapeutic agents for future Phase III trials more meaningful.

In addition to testing the efficacy of various treatments in malignant brain tumor, radiation effects and drug toxicity are being assessed. Data is also being accumulated that will provide a basic understanding of the biology, natural course and peculiarities of the disease entity under investigation.

Work completed has shown that radiation therapy alone and in combination with the nitrosoureas significantly extends the median survival of brain tumor patients. The oncologic effect of corticosteroids is being evaluated in the current Phase III trial.

PROJECT DESCRIPTION:

The Brain Tumor Study Group (BTSG) was established in order to provide a rapid, scientific and controlled method of evaluating chemotherapy in the treatment of malignant gliomas utilizing Phase III type clinical trials. The goals of the BTSG are (1) to increase the number of patients that respond to treatment, (2) prolong the period of remission, and (3) increase the life expectancy of these patients. Since inception, the program has been broadened to include evaluation of radiation therapy as a modality alone and in combination with chemotherapy. Additionally, the BTSG is also conducting Phase II studies in order to make a more meaningful selection of chemotherapeutic agents for future Phase III studies.

Members of the Brain Tumor Study Group have established a cooperative, ongoing and continuous effort providing patients for multi-therapeutic modality studies which utilize a prospective, controlled, randomized study design. Patients diagnosed for the first time as having malignant gliomas are randomized to the Phase III study shortly after definitive surgery. Patients with recurrent gliomas or other types of brain tumors are randomized to the Phase II study.

A paper summarizing the results of the Phase III study evaluating BCNU and radiation therapy alone and in combination is in preparation. Of the 303 patients randomized into the four treatment arms (best conventional care, BCNU, radiation therapy, BCNU plus radiation therapy), 225 were valid study patients. Results of this study indicated that radiation therapy alone and radiation therapy in combination with BCNU significantly increased the survival time of patients with malignant gliomas.

The subsequent Phase III clinical trial designed to evaluate MeCCNU alone and in combination with radiation therapy was closed to accrual but is continuing to treat and follow patients. Of the 467 patients randomized to this study, 456 are evaluable. Final data analysis for this protocol can be completed this period since all the surviving patients have survived beyond the median survival time for the treatment group to which they were randomized. Preliminary analysis of the current data base indicates a median survival of 51 weeks for patients receiving BCNU plus radiation therapy as compared with those receiving radiation therapy alone (37 weeks), MeCCNU alone (23 weeks) and MeCCNU plus radiation therapy (43 weeks).

The currently active Phase III protocol was designed to evaluate both the oncological effect of high dose corticosteroids in brain tumor patients, as well as, survival. Methylprednisolone is being compared with BCNU, procarbazine, and methylprednisolone plus BCNU following definitive surgery and concomitant radiation therapy of patients with malignant gliomas. Accrual has reached the point where the development phase of the next Phase III protocol will be initiated. The study is too preliminary to predict the median survival time for the various treatment arms using the statistical method of Kaplan and Meier, however, preliminary indications are that all treatment arms will be as effective or more effective than the control arm of the protocol, *i.e.*, the best treatment regimen of the previous protocol.

The Phase II study which was testing the effectiveness of adriamycin, dibromodulcitol, procarbazine and streptozotocin in brain tumor patients was closed to accrual. Preliminary results indicate that the median survival time of procarbazine treated patients was marginally better than those of the other drugs being tested. Procarbazine was, therefore, moved into the current Phase III trial. Subsequent analysis of this data base has shown that the median survival time for the streptozotocin treated patients was not statistically different from the patients receiving procarbazine. Streptozotocin was, therefore, moved into the recently activated Phase II protocol for further evaluation.

The currently active Phase II protocol is evaluating streptozotocin, hydroxyurea, epipodophyllotoxin and the combination of MeCCNU plus procarbazine in patients with brain tumors. The protocol design stratifies according to pathology, radiation therapy and the neurological status of the patient at the time of randomization -- factors known to influence survival. It is premature to do a data analysis of this study.

The Brain Tumor Study Group program received a comprehensive review by the DCT-Clinical Trials Review Committee on March 31, 1977. Favorable comments on the scientific and administrative aspects of this program were made by the members of this committee, culminating with the recommendations to continue funding of this effort.

The Fall 1976 meeting of the BTSG was a two-day session consting of a business session in the morning of the first day and a scientific session for the remainder of the meeting. The status and toxicity encountered in the various group protocols was presented and discussed during the business portion of the meeting. The scientific portion of the meeting covered a variety of interesting and pertinent topics related to the investigation of brain tumors. The program was extremely well received by those in attendance.

The Spring 1977 meeting of the BTSG consisted of a morning business session at which the status, results and toxicity of the current protocols were presented and discussed. During the afternoon portion of the meeting, new surgery, radiation therapy and chemotherapy approaches that might be incorporated into future BTSG protocols were presented by the respective session chairmen. The rational, feasibility and other pertinent aspects of these suggested approaches were thoroughly discussed. Further study of these suggested approaches will be made by the BTSG Protocol Design Committee before they are utilized in the development of future protocols.

The BTSG studies continue to serve as a prototype for the cooperative efforts utilizing prospective, controlled, randomized studies for the treatment of various malignancies. The BTSG studies are generating specific, detailed and timely information concerning the efficacy of the various therapeutic modalities utilized, as well as insight into the biological characteristics of the disease entity being investigated. This project is also in keeping with both the long range goal and short term objective of the Division of Cancer Treatment program.

Publications:

Walker, M.D.; et al.*: "The Evaluation of Mithramycin (NSC-24599) in the Treatment of Anaplastic Gliomas", J. of Neurosurg. 44:655-667, 1976.

Mahaley, S.M., Jr., et al.: "Immunobiology of Primary Intracranial Tumors - Part 1: Studies of the Cellular and Humoral General Immune Competence of Brain Tumor Patients", J. of Neurosurg. 46:467-476, 1977.

Levin, V., et al.: "Criteria for Evaluating Malignant Brain Tumor Patients Undergoing Chemotherapy", J. of Neurosurg. In press.

Walker, M.D., et al.*: "An Evaluation of BCNU and/or Radiotherapy in the Treatment of Anaplastic Gliomas (a Cooperative Clinical Trial) for the Brain Tumor Study Group", J. of Neurosurg. In press.

Walker, M.D., et al.: "Therapeutic Studies in the Treatment of Malignant Glioma", National Cancer Institute monograph #46, 1977.

Gehan, E.A.: "Prognostic Factors for Patients with Brain Tumors." National Cancer Institute Monograph No. 46, 1977.

Mahaley, M.S., Burger, P., Vogel, S., Ghatak, N.R.: "Neuropathology of Tissue From Patients Treated by the Brain Tumor Study Group." National Cancer Institute Monograph No. 46, 1977.

Ransohoff, J., Weiss, J.: "Cerebral Spinal Fluid Sterols in the Evaluation of Patients with Gliomas." National Cancer Institute Monograph No. 46, 1977.

SUMMARY REPORT

Clinical Branch, Baltimore Cancer Research Center, DCT, NCI
From July 1, 1976 through September 30, 1977

A number of fruitful projects have been undertaken during this fiscal year and many have produced interesting results already. A Phase I study of nitrous oxide has been started. Phase II studies in lung cancer of levamisole and of intrathecal ThioTepa in meningeal leukemia have been completed. Phase II evaluation of Dianhydrogalactitol and piperazinedione in brain tumors have been completed. Phase II evaluations of ThioTepa, vinblastine, CCNU, and piperazinedione in renal cell carcinoma have also been completed. Additional Phase II studies completed include studies of azotomycin in metastatic sarcoma and adriamycin in mycosis fungoides. A Phase II study of anguidine in colon cancer has been implemented, and a Phase III Study of head and neck cancer is underway. Phase III studies with daunorubcin and cytosine arabinoside in adults with acute nonlymphocytic leukemia have been very rewarding. Seventy percent of such patients irrespectable of age achieve a complete remission with that combination of agents. A Phase III study of methyl CCNU and ftorafur in colon cancer has been completed.

Combined modality studies of Hodgkin's disease continue to show significant advantage for radiotherapy-chemotherapy in patients with stage III A or with earlier stages associated with E sub-stage of the lung. A Phase III study of cytoxan, adriamycin and VP16-213 for oat cell carcinoma of the lung has produced some of the best data obtained with chemotherapy or chemotherapy and radiotherapy for this disease. Approximately 40% of patients have achieved a complete remission with this regimen and it is clear that completed remission is contributing to substantially improved survival. A Phase III study of streptozotocin, cyclophosphamide, adriamycin and bleomycin for advanced refractory Hodgkin's disease has lead to the conclusion that this combination of agents is highly active in patients who are refractory to or relapsing from, MOPP therapy. Studies of this combination in previously

The immunotherapeutic agent MER has been investigated in colon cancer, oat cell carcinoma of the lung melanoma, and previously treated acute leukemia patients. In each situation the agent has been found to be inactive.

Epidemiological investigations of patients with leukemia and lymphoma have suggested that such patients are often linked by prior social contact with common, healthy, intermediaries. These studies continue.

A randomized comparison of cephalothin plus carbenicillin, carbenicillin plus gentamicin, and cephalothin plus gentamicin for the empiric antibiotic treatment of febrile granulocytopenic cancer patients has identified the combination of carbenicillin and gentamicin as superior from both an efficacious and a toxicologic point of view. Newer aminoglycoside antobiotics have been investigated in a similar fashion.

We have discovered that fire proofing material applied to the steel beams in the new hospital building in which the BCRC operates contains aspergillus spores. This has been related to the increased incidence of aspergillus infection immediately after moving into this new facility. Consequently, we have undertaken to decontaminate this fire proofing material and to inform others in similar situations of the potential hazzard such fire proofing material represents for granulocytopenic cancer patients. Treating the fire proofing material with copper-8-quinolinolate has reduced our aspergillus infection rate to zero.

We have undertaken a study involving the treatment of patients with diffuse histiocytic lymphoma in a laminar air flow room. This protection allows us to deliver larger than usual doses of cytoxan, adriamycin, vincristine and prednisone to such patients. Early data indicate that the complete remission rate is high with this approach. However, the regimen appears to be too toxic and is associated with several drug related deaths.

Studies with granulocyte transfusions in febrile granulocytopenic cancer patients have indicated that these transfusions can be beneficial and life saving. Studies are underway to develop methods for increasing the yield of granulocytes from normal donors and studies are in progress to reduce the toxicity of granulocyte transfusion to the host. Studies with autologous frozen platelets in acute leukemia patients indicate that these platelets can be life saving to such patients who have become refractory to HLA compatible platelets from other donors. A study of prophylactic granulocyte transfusion has been started.

Biologic markers of malignant disease such as muramidase, alpha feto protein, carcinoembryonic antigen, Regan isoenzyme, and reverse transcriptase have been studied in some detail and the data correlated with the type and extent of malignant disease.

The massive research data accumulated by the Branch has been prepared for analysis and analyzed by a pilot computerized problem oriented medical system. Physicians, nurses, pharmacists and other related health care personnel take part in the in-put and retrieval of this data.

Various new pieces of equipment have been evaluated at the Baltimore Cancer Research Center during this fiscal year including the acoustic microscope, which is an instrument that utilizes microwaves to produce an image of a microscopic particle. Early evaluations indicate that this instrument may be useful for the rapid examination of hematologic material. A device that allows for storage of frozen platelets under various partial pressures of certain gasses has been developed.

Participation in oncology programs at the two medicals schools in Baltimore and in other hospitals around the community has provided a source of patient material for our studies and has enabled us to bring to the practicing and academic physician in the community, the impact of the therapeutic advances resulting from the DCT program.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 06230-08 COB

PERIOD COVERED
July 1, 1976 through Sept.30, 1977

TITLE OF PROJECT (80 characters or less)

Diagnostic Laparotomy and Splenectomy for Staging Hodgkin's Disease

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Peter H. Wiernik, M.D.	Chief, Clinical Oncology Branch	CL NCI
	Charles H. Diggs, M.D.	Investigator	CL NCI
Others:	John Sutherland, M.D.	Pathology, Univ. of MD Hospital	
	James Ryan, M.D.	Nuclear Medicine, Univ. of MD Hospital	

COOPERATING UNITS (if any)
Pathology, Tumor Surgery, Radiology and Nuclear Medicine Departments,
University of Maryland Hospital, Baltimore, Maryland

LAB/BRANCH
Clinical Oncology Branch

SECTION
Office of the Chief

INSTITUTE AND LOCATION
NCI, NIH Baltimore, MD 21201

TOTAL MANYEARS: 0.5	PROFESSIONAL: 0.5	OTHER: 0

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline k

The purposes of this study have been to determine the value of staging laparotomy on evaluating the accuracy of the extent of abdominal disease at the time of initial evaluation and what influence this has on the initial therapy for patients with early stage Hodgkin's disease. 175 patients with Hodgkin's disease have undergone staging laparotomy and splenectomy. This has revealed that the pre-operative assessment of the extent of abdominal involvement was incorrect in approximately 40% of these cases. The prediction of splenic involvement by Hodgkin's disease was incorrect in one-third of the patients and approximately one-third of abnormal lymphangiograms could not be confirmed at laparotomy. A certain number of cases of involvement of nodes in the porta hepatic region outside the usual irradiation therapy ports were discovered. A small number of patients were also found to have documented liver involvement which had not been suspected by pre-operative evaluation. These findings have altered the therapeutic approaches significantly in a number of patients. The results of these studies have been important in the subsequent design of further therapeutic protocols for the treatment of Stages I-A to III-A Hodgkin's disease.

Accurate determination of extent of lymphomatous involvement with Hodgkin's disease is important for the individual patient's prognosis and therapy. Valid assessment of the efficacy of different therapeutic modalities used in a population with Hodgkin's disease is also dependent upon a precise localization of disease prior to therapy. The purpose of this study is to determine more accurately the extent of abdominal disease at the time of diagnosis and prior to therapy by performing diagnostic laparotomy.

Analysis of 175 patients with Hodgkin's disease who have undergone staging laparotomy and splenectomy has revealed that pre-operative assessment of the extent of abdominal involvement was incorrect in approximately 40% of the cases. Prediction of splenic involvement by Hodgkin's disease was incorrect in 1/3 of the patients, approximately 1/3 of abnormal lymphangiograms could not be confirmed at laparotomy, and a number of cases of involvement of nodes in the porta hepatis region outside the usual radiation therapy ports were discovered. Occult hepatic involvement was discovered in some cases in which liver function tests and physical examination were normal, and others suspected of having liver involvement were shown to have disease confined only to lymph nodes. Theses findings altered therapeutic approaches significantly in many patients.

It was discovered that patients presenting with supradiaphragmatic Hodgkin's disease had occult disease limited to the spleen, splenic hilum and celiac axis nodes in most cases in which abdominal tumor was documented at laparotomy. Extranodal involvement was not seen in any of the 85 patients with supradiaphragmatic clinical Stage I or II Hodgkin's disease and most patients would have had all apparent disease adequately treated had extended field radiation therapy been administered without performing staging laparotomy. Therefore, a subsequent project analyzed the value of performing staging laparotomy in patients with early clinical stage Hodgkin's disease. This present study is now closed to patient accrual.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 06259-05 COB

PERIOD COVERED

July 1, 1976 through September 30, 1977

TITLE OF PROJECT (80 characters or less)

Extension of the Computerized PROMIS System into the Cancer Treatment Area

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Robert Esterhay, M.D.	Senior Investigator	CL NCI
Others:	Paul Chang, M.D.	Senior Investigator	CL NCI
	Amy Hament, R.N.		CL NCI
	William Grove	Pharmacist	CL NCI
	Clarence Fortner	Pharmacist	CL NCI
	William Robinson	Research Programmer	CL NCI
	Carole Rulenz, A.R.T.		CL NCI
	Voila Mae Young, Ph.D.	Head, Microbiology Section	CL NCI
	Peter H. Wiernik, M.D.	Chief, Clinical Oncology Branch	CL NCI
	Michael D. Walker, M.D.	Associate Director, BCRC	CL NCI

COOPERATING UNITS (if any)

PROMIS Laboratory, Department of Medicine, University of Vermont, Burlington, Vermont

LAB/BRANCH

Clinical Oncology Branch

SECTION

Office of the Chief

INSTITUTE AND LOCATION

NCI, NIH Baltimore, Maryland 21201

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
5	5	0

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☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline ke

The Baltimore Cancer Research Center (BCRC) has developed cancer guidance displays (treatment protocols) on the computerized Problem Oriented Medical Information System (PROMIS) in Burlington, Vermont at the University of Vermont School of Medicine, with the aid of a communication linkage between BCRC and PROMIS. The computerized displays for the logic and action of treating cancer problems are defined by the creation and sequencing of the computer display pages or frames. These displays provide an automatic connective device between the four phases of medical action of the Problem Oriented Medical Record. The problem defining sequence determines the specific plan and it is that plan which is in part a protocol or series of protocols for treating a specific cancer problem. The protocol couples the health provider to the protocol specific antineoplastic drug displays which provide feed back loops to the patient's problem list and outstanding orders. It is through the use of these explicit informational and directional displays that the health provider can couple learning with doing while gathering information and treating patients' cancer problems.

To extend the Computerized Problem-Oriented Medical Information System (PROMIS) to the cancer treatment area to allow the development of structured input ("cancer displays") for coupling learning with doing for all personnel who care for individual cancer patients entered on problem specific plans (research protocols).

Methods Employed:

1. The computer terminal being used for this purpose is a touch-sensitive cathode ray tube display device similar to those in use in Burlington, Vermont, where the computer is located. BCRC has been responsible for the installation since February, 1972. All programming and operation of the computer system have been done at the University of Vermont.

2. The basic components of the remote terminal at the BCRC are the same as the local terminals in Burlington, Vermont: display screen, display memory, keyboard, touch screen and communication link to the computer in the PROMIS Laboratory, Burlington, Vermont.

3. The BCRC staff has developed anti-neoplastic drug displays which have been integrated with the displays already on file in Burlington, Vermont, as part of the PROMIS system. Currently over 30,000 displays have been developed by the PROMIS group. The drug information sequences have been up-dated and new sequences have been added.

4. Problem-specific display plans for acute non-lymphocytic leukemia and non-Hodgkin's lymphomas have also been developed which includes a series of protocols for treating these specific problems. The protocol displays couple the user to the protocol specific information and drug ordering display sequences. The problem formulation sequence displays for chronic lymphocytic leukemia and Hodgkin's disease have been developed. Displays for problem-oriented reporting of bone marrow findings are being tested. Problem-specific plans for conventional as well as protocol treatment have been developed for chronic lymphocytic leukemia and Hodgkin's disease. In addition, problem formulation sequences for lung cancer and problem-specific plans for small cell anaplastic carcinoma of the lung are under development.

The computerized displays for the logic and action of treating cancer problems are defined by the creation and sequencing of the computer display pages or frames. These displays provide an automatic connective device between the four phases of medical action of the Problem-Oriented Medical Record. The problem formulation sequence determines the specific plan and it is that plan which is in part a protocol or series of protocols for treating a specific cancer problem. The protocol couples the user to the protocol specific anti-neoplastic drug displays which provide feedback loops to the patient's problem list and outstanding orders. It is through the use of these explicit informational and directional displays that the user is provided guidance while gathering information and treating patients' cancer problems.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 06263-06 COB

PERIOD COVERED
July 1, 1976 through Sept. 30, 1977

TITLE OF PROJECT (80 characters or less)

Protocol for Staging and Treatment of Lymphosarcoma and Reticulum Cell Sarcoma

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Charles H. Diggs, M.D.	Investigator	CL NCI
	Peter H. Wiernik, M.D.	Chief, Clinical Oncology Branch	CL NCI
Others:	Robert Slawson, M.D.	Acting Director, Division Radiation Therapy, University of Maryland Hospital	

COOPERATING UNITS (if any)

Radiotherapy Service and Surgical Service,
University of Maryland Hospital, Baltimore, Maryland 21201

LAB/BRANCH
Clinical Oncology Branch

SECTION
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INSTITUTE AND LOCATION
NCI, NIH Baltimore, Maryland 21201

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.5	0.5	0

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline k

At the time of presentation, non-Hodgkin's lymphomas are frequently disseminated. There exists, however, a number of patients with localized disease following completion of all clinical and pathologic staging procedures. It is in this group of patients that radiotherapy is most useful. However, the question of whether adjuvant chemotherapy following radiation therapy is of any benefit is as yet unanswered. Patients who are found to be pathologically Stage I and II non-Hodgkin's lymphomas following a variety of staging procedures including staging laparatomy, are randomized to receive either extended field irradiation alone, or extended field irradiation followed by six courses of chemotherapy using Cytoxan, vincristine and prednisone. Thirteen patients whose staging laparotomies were entirely negative have been randomized to one or the other of the treatment arms. Too few patients have been accrued at this time to determine the value of adjuvant chemotherapy in this setting.

1. To assess the value of surgical staging in patients with apparently localized non-Hodgkin's lymphomas.

2. To determine whether adjuvant chemotherapy will prolong remissions in patients with pathologically localized lymphomas treated with radiation therapy.

Patients with malignant non-Hodgkin's lymphomas will undergo a variety of staging procedures. Those who remained clinically localized following non-invasive procedures will undergo staging laparotomy to detect occult foci of intra-abdominal disease. Patients who are found to be pathologic Stage I or II will then be randomized to receive (a) radiation therapy alone, or (b) radiation therapy followed by six courses of combination chemotherapy using Cytoxan, vincristine and prednisone. Patients who are found to have advanced disease by these staging procedures will be treated with chemotherapy alone on a different regimen. Of the patients undergoing all staging procedures, there have been 13 who have been found to have pathologic Stage I or II. At this time, there are too few patients in either of the study arms to determine the value of adjuvant chemotherapy.

Staging laparotomy as an additional diagnostic test to determine the extent of disease in the non-Hodgkin's lymphomas should allow better patient selection for attempts at curative radiation therapy in those patients who are found to have truly localized disease. Adjuvant chemotherapy conceivably may offer prolonged disease-free and overall survival.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 06275-08 COB

PERIOD COVERED
July 1, 1976 through September 30, 1977

TITLE OF PROJECT (80 characters or less)
Cell Transfusion Therapy

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Charles A. Schiffer, M.D.	Senior Investigator	CL NCI
	Joseph Aisner, M.D.	Senior Investigator	CL NCI
	Peter H. Wiernik, M.D.	Chief, Clinical Oncology Branch	CL NCI
Other:	Francis Sanel, M.D.	Investigator	LPH NCI
	Karen Lichtenfeld, M.D.	Clinical Associate	CL NCI
	Martin Helrich		
	Lucy Barrett		

COOPERATING UNITS (if any)
Baltimore Red Cross, Community Blood and Plasma Service, Baltimore and Dr. Paul Terasaki, University of California, Los Angeles; and the University of Maryland, Departments of Pathology and Anesthesiology

LAB/BRANCH
Clinical Oncology Branch

SECTION
Office of the Chief

INSTITUTE AND LOCATION
NCI, NIH Baltimore, Maryland 21201

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
8	4	4

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☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline k

In the past year, platelet and granulocyte support of alloimmunized patients has improved considerably as a result of the creation of a HLA typed donor file. Analysis of these transfusions is in progress in an attempt to determine the relative antigenicity of different cross-reactive HLA antigens and to define certain patterns of alloimmunization. HLA matched platelets have also been cryopreserved using DMSO, and in particular a large number of frozen autologous platelet transfusions obtained from patients in leukemia have been administered with in vivo recoveries of between 50-60%. Ultrastructural studies of the frozen platelets and a model system which measures release of LDH are being utilized to improve upon these results. We have continued to administer granulocytes as early in the course of the infectious illness as possible and have accumulated further evidence of the efficacy of granulocyte transfusion. A protocol studying the prophylactic use of granulocyte transfusion which will also study certain aspects of the alloimmunization process will also be implemented. Experiments designed to improve elution of the granulocytes from nylon fibers have been carried out. Anaesthetic agents are capable of reversing granulocyte adherence to fibers and, under the proper conditions, do not cause significant, irreversible damage to the granulocytes. Studies about the effect of these drugs on platelet function as well as studies on corticosteroid related changes in platelet function are in progress.

The Cell Transfusion Program is organized to provide platelets and granulocytes for the treatment and prophylaxis of hemorrhagic and infectious complications in patients with leukemia, lymphoma and other types of solid tumors receiving aggressive chemotherapy. In addition, an active laboratory focusing on various aspects of platelet and granulocyte function is in operation.

I. Platelet Transfusion and Studies:

It is still our policy to transfuse random donor platelets obtained at the Baltimore Red Cross and the Community Blood and Plasma Service prophylactically to patients with platelet counts less than 20,000/ml. Approximately 8 to 9,000 units of platelets are transfused per year of which about 1500 units are obtained at the BCRC. Most of the latter units are utilized for the support of alloimmunized patients and a large computerized donor file, which now comprises of about 900 HLA matched donors, has been created. We are presently beginning a detailed analysis of the results of these HLA matched transfusions in an effort to determine the relative importance of different cross-reactive HLA antigens, the relative immunogenesity of different HLA antigens, and an analysis of patterns of histocompatibility antibody development and elimination. In some cases, specific platelet cross-matching using platelet aggregometry has also been used.

The platelet collection studies have involved serial determinations of platelet size in various aliquots obtained by either the traditional "bag" technique or using the Model 30 Blood Processor. Preliminary evidence suggest that at least in patients with leukemia, a population of large (possibly younger and more functional) platelets can be harvested by going deeper into the red blood cell layer.

Platelet cryopreservation, with particular attention paid to autologous platelets obtained by plateletpheresis of patients with leukemia in remission has remained an active interest in the laboratory. Techniques have been modified and results have improved significantly. An analysis of the last 40 autologous transfusions reveals an one hour recovery of 50% which compares quite favorably with results obtained during fresh platelets. Furthermore, a number of patients have been supported entirely through periods of remission maintenance and reinduction therapy using solely their autologous platelets and one particularly important patient for whom no donors could be located has been supported entirely with his own platelets with platelet recoveries greater than 75%. In an effort to improve these results, experiments in the laboratory using small aliquots of platelets are in progress. A system which measures simple release of cytoplasmic enzyme, lactic dehydrogenase has been developed which is presently being utilized to test modifications of the freezing and thawing technique. Using this system, it has been possible to document the cryoprotective value of DMSO and it has also been shown hydroxyethyl starch seems to be ineffective as a cryoprotective agent for platelets. Ultrastructural studies of platelets are also in progress and to date have revealed that approximately 50-60% of the platelets frozen and thawed maintain discoid shape (strikingly similar to the *in vivo* recovery) while the remainder of cells appear to be less viable. Attempts are being made to correlate these observed changes with more easily measurable changes in platelet size.

As an outgrowth of these studies with local anesthetics described below, we have also measured the effects of local anesthetic agents on platelets. Dose related changes in size, platelet factor-3 release and reversible impairment of aggregation have been noted. The change in shape appears to be related to a diminution in the number of circumferential microtubules. Experiments are in progress to determine whether microtubules can be reformed after the cells are washed free of the anesthetics. As a clinical correlate to this, studies are in progress to determine whether general anesthetic agents impair platelet function when administered during the course of surgery. These studies are in progress and no results are available as yet.

II. Granulocyte Transfusion:

It is now the policy to administer granulocytes as early in the course of serious infection as possible in patients in whom early bone marrow recovery is not anticipated. In particular, patients with pneumonia and/or gram-negative bacteremia are candidates for early intervention with granulocytes obtained either by the Model 30 Blood Processor or by filtration leukopheresis. Joint rounds are now made twice a week with the Infectious Disease Section and decisions about antibiotics and granulocyte usage (and evaluation of clinical response) are discussed at these times. This has resulted in a more uniformed application of granulocyte transfusion and in excellent clinical follow-up. Presently, one Haemaonetics Model 30 apparatus and two filtration leukopheresis set-ups are available for granulocyte collection.

Because of the increased incidence of serious transfusion reactions noted following transfusion of granulocytes obtained by filtration leukopheresis, series of studies has been carried out in order to improve the elution of these cells from the fibers. It has been demonstrated that the local anesthetic agents are capable of eluting granulocytes adherent to nylon fibers and that these cells appear to be more normal in morphology than cells obtained using traditional methods. It was also demonstrated that in proper concentrations, the drugs do not effect granulocyte viability as assessed by dye exclusion and enzyme leakage, markedly alter the cell surface of granulocytes producing a change in shape which is reversible upon removal of the drugs, and reversibly and totally inhibit granulocyte phagocytosis and adherence. Greater than 95% of the drug can be removed by a single centrifugation and resuspension suggesting that this methodology may be clinically applicable. Similar studies were also attempted with procaineamide and levamisole were unsuccessfull in eluting cells from the fibers. Experiments attempting to modify these results at different temperatures and with different concentrations of the drugs are in progress. A randomized control trial of prophylactic granulocyte transfusion will also be initiated in the near future. The primary intent of this protocol is to test whether granulocytes administered with routine platelet transfusions on an every other day schedule will serve to decrease the incidence of infection in patients with leukemia receiving initial induction of chemotherapy. In addition, we plan to carefully study the development of alloimmunization to platelet and leukocyte antigens and hope to derive significant data about the pattern of antibody development when only a limited pool of donors is used from the very beginning of transfusion support. Because all donors will be premedicated with corticosteroids in an effort to increase the granulocyte yield, studies are in progress dealing with the possible effect of corticosteroid on platelet function because platelets are collected simultaneously with the granulocytes. Preliminary evidence suggest that there is no significant effect of corticosteroids on platelet aggregation.

Recently a procedure for granulocyte collection which involves the addition of hydroxyethyl starch, two units of whole blood followed by gravity sedimentation of red cells has been described. Studies will be undertaken to verify these conclusions and to possibly improve on the results by variations in temperature, addition of centrifugation steps, etc.

Publications:

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 06276-04 COB

PERIOD COVERED
July 1, 1976 through September 30, 1977

TITLE OF PROJECT (80 characters or less)

The Chemotherapy of Metastatic Testicular Carcinoma

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Davis M. Hahn, M.D.	Senior Investigator	CL NCI
	Peter H. Wiernik, M.D.	Chief, Clinical Oncology Branch	CL NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH
Clinical Oncology Branch

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Office of the Chief

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NCI, NIH Baltimore, MD 21201

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.5	0.5	0

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☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline k

The addition of bleomycin to 5-FU, Cytoxan, methotrexate and vincristine had been shown in preliminary evaluations of this study to markedly enhance the efficacy of this combination of agents against metastatic testicular carcinoma. More recently these patients have been treated with adriamycin in combination with cis-diaminedichloroplatinum. A pilot study with these drugs demonstrated greater than 50% response rate. More recently adriamycin in doses of 60 mg/m^2 and cis-diaminedichloroplatinum in doses of 75 mg/m^2 day 1 and day 8 had been given. Five patients have been treated. There have been two patients who initially had partial responses who have failed therapy and one patient who is in complete remission 10 months into therapy. Another patient did not have any measurable disease to follow, but is free of disease after 6 courses. Side effects of the regimen have been significant. Four of five patients have had dose reduction in cis-platinum and 3 of the 5 had to have dose reduction of adriamycin. Weight loss has been a mild problem with less than 5% total body weight lost. One patient had renal failure with creatinine rising to 9 but returning to 2.4. This patient has been able to continue dis-platinum in decreased dose. The combination of adriamycin and cis-platinum has been proven efficacious, but the protocol will have to be altered because of the toxicity.

PHS-6040
(Rev. 10-76)

Objectives are to assess the efficacy of various anti-tumor chemotherapy agents in metastatic testicular carcinoma. Patients were previously randomized between a combination of drugs and 5-FU, Cytoxan, methotrexate, vincristine plus or minus bleomycin given 12 hours after vincristine. Patients in this protocol were then given adriamycin and cis-platinum. More recently patients have all been given adriamycin dose of 60 mg/m^2 IV push day 1 and cis-platinum 75 mg/m^2 IV infusion over 6 hours preceded and followed by hydration lasix and mannitol.

Results in the previous study: 29 patients were entered. Objective tumor regression occurred in 20 patients. There was no significant difference between the 4 and 5-drug regimen. Toxicities were equal.

Presently 5 people have been treated with adriamycin and cis-diaminedichloroplatinum. One patient had no measurable disease to follow and is non-evaluable, and one patient just recently has been started on protocol. 3 patients have been evaluated, 2 initially received partial responses lasting 1 and 2 months respectively but have failed chemotherapy. A third patient, although having severe reversible nephrotoxicity is presently in complete remission 8 months later. Three of the 5 patients had to have dose reduction in adriamycin and 4 of 5 patients had to have dose reduction in cis-platinum because of myelosuppression. Additionally, all patients had to have delay in therapy because of myelosuppression. It is our assessment at present that the drugs are active in testicular carcinoma, but because of severe myelosuppression, they have had to be delayed in re-treatment and thus have allowed the tumor to grow. As a result, patients will now be given the following doses: 40 mg/m^2 of adriamycin on day 1 and 60 mg/m^2 of cis-platinum on days 1 and 8.

Publications:

1. Stechmiller, B.K., Wiernik, P.H., Shin, M. Satterfield, J.: Maturation of metastatic charatocarcinoma following chemotherapy to a mass pathologically indistinguishable from a mediastinal enteric cyst. Chest 69:697-700, 1976.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 06277-06 COB

PERIOD COVERED

July 1, 1976 through September 30, 1977

TITLE OF PROJECT (80 characters or less)

Chemotherapy of Adult Acute Leukemia

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Paul Chang, M.D.	Senior Investigator	CL NCI
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Others:	Stephen Schimpff, M.D.	Head, Section of Infection Research	CL NCI
	Charles Schiffer, M.D.	Senior Investigator	CL NCI
	Joseph Aisner, M.D.	Senior Investigator	CL NCI
	Linda Edwards, R.N.		CL NCI
	Mary Scoltock, R.N.		CL NCI
	Viola Mae Young, Ph.D.	Head, Microbiology Section	CL NCI

COOPERATING UNITS (if any)

Clinical Biochemistry Branch, BCRC

LAB/BRANCH

Clinical Oncology Branch

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NCI, NIH Baltimore, Maryland 21201

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline ke

A seven day continuous intravenous infusion of cytosine arabinoside in association with a three day schedule of intermittent daily doses of daunorubicin has been shown to be highly effective chemotherapy for the induction of remission in adults with acute non-lymphocytic leukemia. A complete remission rate of 65% is obtained with this combination. Serum muramidase levels appear to accurately reflect disease activity in a large proportion of patients with acute non-lymphocytic leukemia. Carcinoembryonic antigen and serum immunoglobulin concentrations do not appear to accurately reflect disease activity. Similarly, biochemical testing of leukemic bone marrow cells does not allow for selection of favorable groups of patients to be treated with cytosine arabinoside (kinase-deaminase ratio) or daunorubicin alone (daunorubicin reductase level).

Reverse transcriptase has been identified in the bone marrow cells of leukemia patients prior to treatment and in morphologically normal peripheral cells during complete remission. One interpretation of this data is that morphologically and functionally normal granulocytes in the peripheral blood of leukemia patients in complete remission may be derived from leukemic cells.

PHS-6040
(Rev. 10-76)

I. Therapy:

A. Use of Biochemical Predictors of Disease Activity

Preliminary reports suggest that the ability to induce complete remission of acute non-lymphocytic leukemia with cytosine arabinoside and daunorubicin can be predicted by biochemical testing of bone marrow cells. Using the kinase-deaminase ratio to select patients to be treated with cytosine arabinoside and the daunorubicin reductase level to select patients to be treated with daunorubicin alone, we have attempted to give previously untreated patients with acute non-lymphocytic leukemia the drug which their biochemical tests predict they should be most sensitive to. Three patients with high ("favorable") kinase-deaminase ratios and low ("unfavorable") daunorubicin reductase concentrations received cytosine arabinoside alone without achieving complete remission. Eight patients with higher daunorubicin reductase levels and low kinase-deaminase ratios received daunorubicin alone. Two patients achieved complete remission and one patient had a good partial remission. Forty additional patients had low kinase-deaminase ratios and low daunorubicin reductase concentrations, and were treated with a three day schedule of daunorubicin plus a seven day schedule of cytosine arabinoside. Twenty-six patients (65%) achieved complete remission.

No relationship could be discerned between concentrations of kinase, deaminase, kinase-deaminase ratio, or daunorubicin reductase and attainment of complete remission, duration of complete remission, or survival from therapy in any of the treatment groups. This study did not confirm any usefulness of these enzyme assays in predicting attainment of complete remission with either cytosine arabinoside or daunorubicin alone or the combination of both drugs together.

B. Maintenance Therapy for Remission in Adults with Acute Non-Lymphocytic Leukemia.

Intensive courses of high dose methotrexate with citrovorum rescue are being utilized, along with other drugs, for maintenance of first complete remissions. Although some relatively long term remissions have been achieved, it is too early to completely evaluate this regimen.

II. Biochemical Indicators of Disease Activity in Acute Non-Lymphocytic Leukemia:

A. Serum and Urine Muramidase Studies

We continue to confirm the utility of serum muramidase levels in differentiating between acute monocytic, myelomonocytic, and myelocytic leukemia. In addition, we continue to observe that serum muramidase levels accurately reflect disease activity in a large proportion of patients with acute non-lymphocytic leukemia. It has also become evident that patients with initially elevated serum muramidase levels who achieve complete remission and normal serum muramidase levels have a longer complete remission than those who achieve hematologic and clinical remission but maintain elevated serum muramidase levels.

B. Carcinoembryonic Antigen

We have observed that approximately 30% of adult acute non-lymphocytic leukemia or acute lymphocytic leukemia patients have abnormal levels of carcinoembryonic antigen in their serum. Unfortunately, these levels do not correlate well with disease activity. Most patients undergoing therapy have a rise for weeks or months in their plasma CEA whether or not they respond to therapy. In addition, when patients relapse the presence or absence of CEA in their plasma at that time does not correlate with findings at the time of presentation.

C. Serum Immunoglobulin Concentrations in Patients with Acute Non-Lymphocytic Leukemia

We have measured quantitatively serum IgA, IgD, IgE, and IgM in patients with acute non-lymphocytic leukemia prior to therapy, during therapy, during remission and relapse. Most patients with acute non-lymphocytic leukemia present with decreased levels of IgG in their serum. When complete remission is achieved these levels become normal.

D. Reverse Transcriptase in Leukemic Cells

This enzyme of presumed viral origin is being looked for in cells and plasma from our leukemia patients by Dr. Sol Spiegelman, New York. Results are preliminary at this point. Fifty patients have been studied prior to therapy and the enzyme was present in the white cells of each. In addition, most patients in complete remission continue to have the enzyme detectable in their white cells. We are now looking for the enzyme in the various fractions of the buffy coat of patients in remission and in autopsy specimens.

E. Cell-Mediated Immunity in Acute Non-Lymphocytic Leukemia

Cell-mediated immunity in acute non-lymphocytic leukemia, either at initial presentation or in subsequent relapse, was determined among 98 patients by recall-antigen skin testing and in 67 patients by attempted sensitization and challenge with dinitrochlorobenzene (DNCB). The leukemic patient's subsequent prognosis in terms of attainment of complete remission of his leukemia, duration of remission and survival could not be correlated with the pre-therapy demonstration of intact delayed cutaneous hypersensitivity.

Summary of Major Findings:

1. Daunorubicin and cytosine arabinoside given in intensive courses continue to produce a complete remission rate superior to that of other regimens tested at this institution or elsewhere.

2. Serum muramidase levels are useful in following disease activity in adult acute non-lymphocytic leukemia.

3. Biochemical testing of leukemic bone marrow cells does not allow for selection of favorable groups of patients to be treated with cytosine arabinoside (kinase-deaminase ratio) or daunorubicin alone (daunorubicin reductase level).

4. Carcinoembryonic antigen is present in increased amounts in the minority of patients with acute non-lymphocytic leukemia. Serial determinations, however, are useless in following disease activity.

5. Cell-mediated immunity in leukemia patients prior to therapy may not be important in determining response.

6. The presence of reverse transcriptase in the white cells of leukemia patients in remission may indicate the necessity for further induction therapy. It may also suggest that "normal" granulocytes present during remission represent matured leukemic cells.

Publications:

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 06280-05 COB

PERIOD COVERED
July 1, 1976 through September 30, 1977

TITLE OF PROJECT (80 characters or less)

Combination Chemotherapy for Advanced Hodgkin's Disease

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Charles H. Diggs, M.D.	Investigator	CL NCI
	Peter H. Wiernik, M.D.	Chief, Clinical Oncology Branch	CL NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH
Clinical Oncology Branch

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NCI, NIH Baltimore, Maryland 21201

TOTAL MANYEARS: 0.5	PROFESSIONAL: 0.5	OTHER: 0

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☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline k

Fifty patients with advanced Hodgkin's disease were treated with a combination of cyclophosphamide, vinblastine, procarbazine and prednisone (CVPP) in an every three week regimen. Patients who achieved complete remission were randomized to receive maintenance therapy consisting of monthly alternating CCNU and vinblastine or to followed on no therapy. Thirty-one patients (62%) achieved compelte remission with the fewest remissions being seen (3 of 8) in those patients who had previously received both chemotherapy and radiation therapy. Maintenance therapy to date has not significantly prolonged remission duration or survival. Patients who receive more than six courses of induction therapy have had longer remissions and fewer relapses than those receiving only six courses regardless of maintenance therapy status. CVPP is an effective regimen at inducing remissions in advanced Hodgkin's disease. Maintenance therapy has not yet been shown to be beneficial.

PHS-6040
(Rev. 10-76

To determine if a combination of cyclophosphamide, vinblastine, procarbazine and prednisone (CVPP) is effective at inducing remissions in advanced Hodgkin's disease regardless of prior treatment status, and to assess the effects of maintenance therapy with CCNU and vinblastine on remission duraiton and survival.

Fifty-nine patients have been entered on study which was closed to further entries on December 31, 1975. Fifty patients are considered evaluable, 27 previously treated and 23 having received no prior therapy. Overall, 31 patients achieved a complete remission; 15/19 of those previously treated with irradiation only, 3/8 of those having previously received irradiation and chemotherapy and 13/23 of those without prior therapy have achieved remissions.

Myelosuppression was the major toxicity with leukopenia being seen more frequently than thrombocytopenia.

As of January 1, 1977, the 16 patients receiving maintenance therapy have not had significantly longer durations of remissions or durations of survival than the 14 patients not being maintained. There have been 11 relapses, five while maintenance therapy was being given.

Patients receiving greater than six courses of induction therapy have had longer remisison duration and fewer relapses regardless of whether maintenance therapy was given or not.

CVPP is an effective induction regimen for advanced Hodgkin's disease regardless of prior treatment status. Maintenance therapy has not yet shown any significant prolongations of remission or survival. This project is now closed.

Publications:

1. Diggs CH, Wiernik PH, Levi JA, and Kvols LK: Cyclophosphamide, vinblastine, procarbazine and prednisone with CCNU and vinblastine maintenance for advanced Hodgkin's disease. Cancer 39, 1977.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 06293-06 COB

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)
A Teaching and Consultation Service in Medical Oncology

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Peter H. Wiernik, M.D.	Chief, Clinical Oncology Br.	CL NCI
Other:	Robert J. Esterhay, M.D.	Senior Investigator	CL NCI
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	Charles Diggs, M.D.	Senior Investigator	CL NCI
	David Van Echo, M.D.	Senior Investigator	CL NCI
	Karen Lichtenfeld, M.D.	Senior Investigator	CL NCI

COOPERATING UNITS (if any)
University of Maryland School of Medicine, Johns Hopkins University School of Medicine Greater Baltimore Medical Center, and the University of Missouri at Kansas City School of Medicine

LAB/BRANCH
Clinical Oncology Branch

SECTION
Office of the Chief

INSTITUTE AND LOCATION
NCI, NIH, Baltimore, Maryland 21201

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☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline k

The dissemination of information concerning the latest techniques in the diagnosis and therapy of cancer patients is of paramount importance in advancing the attack on this disease. By training potential physicians early during their student and house officer years, such information will be brought to the community in a more orderly and useful way than is sometimes possible. By seeing patients in consultation, we have the opportunity to educate the patient's physician as to modern techniques of management for a given neoplastic disease. These teaching functions that we engage in are important in elevating the level of care available to patients with cancer in this area.

PHS-6040
(Rev. 10-76)

The objectives are to teach the theory and practice of medical oncology to medical students and house officers; to acquaint such individuals with newer aspects of chemotherapy, radiotherapy, and surgery as they apply to the management of neoplastic diseases; and to provide a consultative service for difficult patients with neoplastic diseases for other physicians in the Baltimore area.

During their second year, medical students from the University of Maryland are assigned to this service on a regular basis for their physical diagnosis class. Students from the University of Maryland and Johns Hopkins are assigned here during elective periods for a clinical clerkship in medical oncology. In addition, house officers from the Greater Baltimore Medical Center and the University of Missouri at Kansas City School of Medicine have been assigned here for elective periods of their house officer year. University of Maryland Assistant Residents in Medicine rotate through the Clinical Oncology Branch on a regular basis. Physicians in the community refer patients here for out-patient consultation. If the patient meets the qualifications for one of our protocol studies, he is accepted in transfer and admitted to BCRC. If not, the patient is completely evaluated as an out-patient and returned to his physician with specific recommendations for therapy.

Students and house officers who have rotated through the BCRC are recognizing that medical oncology is a bona fide subspecialty of internal medicine. They have learned that patients with neoplastic diseases require special care based on special knowledge. It has been suggested by some local private physicians that the general care of patients with neoplastic diseases in the community has advanced because of our consultation service.

The dissemination of information concerning the latest techniques in the diagnosis and therapy of cancer patients is of paramount importance in advancing the attack on this disease. By training potential physicians at this time, early during their student and house officer years, such information will be brought to the community in a more orderly and useful way than is sometimes possible. By seeing patients in consultation, we have the opportunity to educate the patient's physician as to modern techniques of management for a given neoplastic disease. Both of these teaching functions we engage in are of significant import in elevating the level of care available to patients with cancer in this area.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 06295-05 COB

PERIOD COVERED

July 1, 1976 through September 30, 1977

TITLE OF PROJECT (80 characters or less)

Census: A Remote Access Patient Information Storage and Retrieval System

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	William L. Robinson	Research Programmer	CL NCI
	Larry Kenney	Research Programmer	CL NCI
	Robert J. Esterhay, M.D.	Senior Investigator	CL NCI

COOPERATING UNITS (if any)

Section of Infection Research, Nursing Service, Section of Microbiology, Clinical Research Pharmacy, Administrative Officer, and Clinical Oncology Branch, BCRC NCI, NIH

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline k

The CENSUS system is a set of computer programs which enables the storage and retrieval of a data base of information on cancer patients treated at the Baltimore Cancer Research Center. The computer programs and the data base are accessed from remote terminals connected to a commercial time-sharing computer facility. Structured data input programs facilitate the addition of new information to the data base. A flexible retrieval program produces patient listings sorted by patient name, cancer diagnosis, attending physician, status, and/or research protocols. A medical secretary updates the data base daily and then retrieves a CENSUS report which lists all patients currently on the Clinical Oncology inpatient service and Clinical Oncology clinic visits made that day. A general purpose information management software library performs data base manipulations and retrievals which previously required the development of special purpose computer programs. Several programs have been written to produce administrative and accounting tabulations of the data stored on the system. The system has been in operation since August of 1971, and the data base currently contains records for over 1400 cancer patients.

OBJECTIVES:

1. To compile, update, and retrieve from a data base of medically relevant information on cancer patients that is centralized, readily accessible and maintained by a single non-technical individual.

2. To generate a daily report on all inpatients and their locations, along with their vital statistics and current therapy data to be distributed to all involved in the tasks of clinical research, patient care and administration.

3. To develop flexible data retrieval programs which generate a variety of useful patient summaries keyed on any of several patient parameters.

METHODS EMPLOYED:

1. Information is compiled and recorded daily in a data input log by a medical secretary.

2. A computer program accepts the input datum from the secretary, checks for a logical content and accuracy, and records it on the data base.

3. A second computer program generates a Daily Census Report which reflects the updated status of the data base.

4. The report is then distributed to the scientific and professional staff, remote contracting laboratories and administrative office.

5. The programs and data base are executed and stored on a commercial time-sharing service.

6. Mass memory disc files and remote terminals from the storage and access mediums.

The Daily Census Report has been utilized by all sections and services of the Cancer Center.

1. Admissions Officer: used to assign new patients to their physicians and to distribute the patient load evenly among the physicians.

2. Clinical Associates: used at evening sign-out rounds as a checklist of patients with special problems that may require extra care overnight.

3. Clinical Oncology Branch: the master retrieval programs are most useful to the physician performing a chart review to evaluate treatment protocols and who requires listings of the patient population which are sorted by status, cancer diagnosis, research protocol, etc.

4. Clinical Research Pharmacy Service: used to fill the unit drug cart patient trays.

5. Section of Microbiology: provides the laboratory staff and outside contracting laboratories with relevant information on patients whose cultures are being analyzed. The permanent records of all beds a patient has occupied provide a useful epidemiological tool in detecting the spread of infectious organisms. The data base is also accessed by computerized microbiology reporting systems at the BCRC (see Project Report Z01 CM 06039-05 BCRC).

6. A general purpose information management software library now performs data base manipulations and retrievals which previously required the development of special purpose computer programs.

7. Several programs have been written to produce administrative and accounting tabulations of the data stored on the system.

The content and flexibility of the retrieval summaries generated by the Census System have provided the Research Center with a valuable "index" to patient records. The daily routine of update and audit have eliminated the inaccuracies and incompleteness of previous disjoint and ill-kept data bases. Five years of continuous operation of the Census System have provided BCRC with a substantial data base of information on cancer patients. Future developments will concentrate on effective utilization of the information thus obtained. These developments will be in the form of programs to correlate census data with data compiled by other data systems at the BCRC.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 06299-04 COB

PERIOD COVERED
July 1, 1976 through September 30, 1977

TITLE OF PROJECT (80 characters or less)

Therapy of Patients with Metastatic Sarcoma

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Paul Chang, M.D.	Senior Investigator	CL NCI
	Peter H. Wiernik, M.D.	Chief, Clinical Oncology Branch	CL NCI
Others:	Nicholas Bachur, M.D., Ph.D.	Chief, Clinical Biochemistry Branch	LPH NCI

COOPERATING UNITS (if any)

Clinical Biochemistry Branch, BCRC

LAB/BRANCH
Clinical Oncology Branch

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline k

The combination of adriamycin and actinomycin-D is being used to treat patients with inoperable or metastatic sarcoma. Results thus far suggest that the combination may increase the percent of objective remissions in these patients. It is as yet too early to tell whether remission duration or survival have been improved. A disease oriented phase II study of azotomycin reveals no significant activity of this drug in patients with these tumors, and significant, almost intolerable, toxicity was noted.

Cerebral metastases were identified either clinically or at autopsy in a higher than expected proportion of patients with advanced sarcomas who had been treated with adriamycin either alone or in combination with other drugs. Such metastases were especially common in patients with malignant fibrous histiocytoma and rhabdomyosarcoma. It may be that successful therapy of such patients with drugs which fail to penetrate the blood brain barrier results in a higher incidence of cerebral metastases.

PHS-6040
(Rev. 10-76)

A. Adriamycin Plus Actinomycin-D for Advanced Sarcomas:

Adriamycin has now become the cornerstone of therapy of inoperable and metastatic sarcomas, having been identified as the most effective single drug in this group of tumors. It has, however, significant dose limiting toxicity, in that 10-30% of patients who receive more than 550 mg/m^2 (cumulative dose) later suffered irreversible cardiac failure.

Actinomycin-D, an antineoplastic antibiotic with a similar mechanism of action, is known to be an effective palliative drug in approximately 20-30% of patients with advanced sarcomas. It is thus believed to be the second most active single agent for chemotherapy of these tumors. A pilot study combining adriamycin 60 mg/m^2 IV q 3 weeks with actinomycin-D 300 mcg/m^2 IV qd days 8 through 12 has therefore been undertaken to attempt to improve the remission rate and ultimately the survival of patients with metastatic or inoperable sarcomas. The rationale for this schedule was based on our previous demonstration that drug induced hepatic dysfunction could influence the metabolism and therapeutic efficacy of adriamycin. It has also been hypothesized that because of the similarity of mechanism of action of the two agents, giving them simultaneously could be counterproductive.

Sixteen patients have thus far been treated. One patient died before actinomycin therapy; one patient was disqualified because the diagnosis of renal cell carcinoma was subsequently made at post-mortem examination; and one patient is too early to evaluate. Of the remaining 14 patients, there have been two complete responses, two partial remisisons, six improvements and four patients have had no response. Duration of remission ranges from 2+ to 9+ months.

Significant toxicities have included nausea with vomiting, mucositis, leukopenia, thrombocytopenia and anemia. Of interest are the observations that mucositis and myelosuppression appear to be moderately increased in frequency compared with the expected toxicities from either drug given alone, however, mucositis and myelosuppression were not seen concomittantly in any patient. Pharmacokinetic studies have been performed to try to elucidate this observation.

The clinical results with this combination appear at least as good as the therapeutic results using adriamycin alone. There appears to be a definite increase in the number of patients with objective improvements and a mild to moderate increase in toxicity. This study continues to accrue patients and the results of the pharmacokinetic assays are being analyzed.

B. Adriamycin Versus Adriamycin Plus Streptozotocin for Patients with Metastatic Sarcoma:

The clinical part of this study has been terminated. During this study it was noted that at an apparent drug interaction betwen adriamycin and streptozotocin resulted in an impairment of excretion of active adriamycin and metabolites, resulting in greater clinical toxicity to patients without an increased therapeutic effect. Additional pharmacokinetic studies of specific adriamycin metabolites are still underway to attempt to further elucidate the pathogenesis of this interaction.

C. Therapy of Previously Treated Patients:

Azotomycin, a diazo analogue of L-glutamine is an antitumor antibiotic which was reported in early phase II studies to cause greater than 50% tumor regression in four of 14 evaluable patients with sarcomas. Subsequently a technical problem in the manufacture of the drug forced its withdrawal from further testing. Because of this apparent activity in the initial trials, however, it was decided to attempt a tumor specific phase II trial in patients with sarcomas to determine the desirability of obtaining a new source for this chemical. We have, therefore, studied this drug as a single agent in previously treated or relapsed patients. Thirteen patients were treated and only one had a brief tumor regression. Significant toxicity in the form of nausea, vomiting, diarrhea, dehydration and prerenal azotemia as well as myelosuppression were identified in most patients. These were of a severe enough degree that even in the one patient who had an apparent tumor response no further therapy could be tolerated. Further trials with this drug thus do not appear warranted.

D. CNS Metastases in Patients with Sarcomas:

Cerebral metastases from patients with sarcomas are extremely infrequent. Recently, however, it has been noted that such metastases may be increasing in frequency, perhaps as the result of successful therapy and prolongation of life through the use of agents such as adriamycin, which does not penetrate the blood brain barrier. In an attempt to confirm these results, the records of 124 patients with inoperable or metastatic sarcoma have been examined. In 9 of our patients cerebral metastases have been identified, four on the basis of clinical findings and diagnostic procedures, five on the basis of these plus post-mortem documentation. No additional cases of brain metastases were found in asymptomatic patients who were autopsied. Particularly surprising was the high incidence of brain metastases in patients with rhabdomyosarcoma (3/14 patients) and malignant fibrous histiocytoma (2/7 patients). Preliminary statistical analysis seems to indicate a longer median survival for patients who eventually develop brain metastases than for patients who do not develop brain metastases. The median survival of patients after the diagnosis of brain metastases, however, is very brief.

Publications:

1. Chang P, Levine MA, Wiernik PH, and Walker MD: A phase II study of intravenously administered methyl-CCNU in the treatment of advanced sarcomas. Cancer 37:615-619, 1976.

2. Chang P: Management of soft tissue sarcomas: Current status. Amer J Med Sci (in press).

3. Chang P, Wiernik PH: Combination chemotherapy with adriamycin and streptozotocin. I. Clinical results in patients with advanced sarcomas. Clin Pharmacol Therapeu 20:605-610, 1976.

4. Chang P, Riggs CE Jr, Scheerer MT, Wiernik PH, and Bachur NR: Combination chemotherapy with adriamycin and streptozotocin. II Clinical pharmacologic correlation of augmented adriamycin toxicity caused by streptozotocin. Clin Pharmacol Therap 20:611-616, 1976.

5. Chang P: Progress in the treatment of osteosarcoma. Med Clin NA (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01-CM 06913-04 COB

PERIOD COVERED
July 1, 1976 through Sept. 30, 1977

TITLE OF PROJECT (80 characters or less)

Bronchial Brushing for Pulmonary Infiltrates in Patients with Cancer

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Joseph Aisner, M.D.	Senior Investigator	CL NCI
Others:	Stephen C. Schimpff, M.D.	Head, Section of Infection Research	CL NCI
	Viola M. Young, Ph.D.	Head, Section of Microbiology Research	CL NCI
	Peter H. Wiernik, M.D.	Chief, Clinical Oncology Branch	CL NCI

COOPERATING UNITS (if any)

Research Microbiology, BCRC,
Pathology and Radiology Departments, University of Maryland Hospital

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1/2	1/2	0

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline k

Since the immunologically compromised patient frequently develops pneumonias which are difficult to diagnose, aggressive diagnostic techniques are warranted. This study established a method of diagnosing unexplained pulmonary infiltrates in an orderly fashion, utilizing transtracheal bronchial brushing, fibroptic bronchoscopy, or percutaneous lung biopsies when other methods have failed. Forty-nine patients with diffuse or nodular infiltrates have undergone such procedures. Thirteen patients were not clinically infected; eight proved to have pulmonary metastases; four proved to have interstitial fibrosis; and one proved to have a collapsed lobe. Thirty-six patients were clinically presumed infected. Twenty-three were proven to have pulmonary infection. Nineteen of the 23 were etiologically documented by these procedures. Thirteen of the 36 patients had no etiology established by any method, although 10 of these 13 patients were receiving broad spectrum antibiotics at the time of the procedure. The diagnosis included a variety of microorganisms: bacterial, protozoal, and fungal. Significant but not fatal complications occurred and were acceptable risks for the patient population. There was no mortality associated with the procedures, and despite pancytopenia, bleeding was a problem in only two patients.

PHS-6040
(Rev. 10-76)

Project Description: Z01 CM 06913-04 COB

The immunologically compromised and myelosuppressed cancer patient frequently develops pulmonary complications, especially infections. Such pneumonias are a contributing factor in the demise of many of these patients. A major reason for the high mortality lies in difficulty of establishing an etiological agent and thereby providing appropriate antimicrobial therapy. Since there is an established low yield of noninvasive culture techniques for pneumonia when occurring in such patients, more aggressive diagnostic techniques are warranted. Myelosuppressed patients frequently carry gram negative organisms and fungi in their naso-oropharynx which will contaminate expectorated sputum. Furthermore, such patients rarely produce sputum spontaneously. The purpose of this study is to establish a method for diagnosing unexplained pulmonary infiltrates when conventional methods such as sputum induction and transtracheal aspiration have been unsuccessful. This study utilizes a transtracheal approach with selective bronchial brushing, peroral fibroptic bronchoscopy, and percutaneous lung biopsies when other methods have failed.

As of March, 1977, 49 patients with either diffuse or nodular pulmonary infiltrates have undergone transtracheal bronchial brushing. Conventional methods including transtracheal aspiration failed to establish a diagnosis in each case. Thirteen patients were not considered clinically infected. Eight of them proved to have pulmonary metastases of which only one was diagnosed by this technique. Four patients for whom no diagnosis was obtained by brushing subsequently proved to have interstitial fibrosis and one additional patient had a collapsed lobe. Thirty-six patients were clinically presumed infected. Ultimately, 23 of these 36 patients were proven to have pulmonary infection, and 19 of the 23 were etiologically documented by brushing. For 13 of the 36 presumed infected patients, no etiology could be established by any method. Ten of these 13 patients were receiving broad spectrum antibiotics at the time of the procedure. Diagnosis established by the brushing procedure included pneumonias involving such organisms as _Aspergillus_, _Mucorales_, _Torulopsis glabrata_, _Staphylococcus aureus_, _Mycobacterium tuberculosis_, _Pneumocystis carinii_, as well as aerobic and anaerobic _Streptococci_. In addition, one case of metastatic sarcoma was identified by cytology. Significant but nonfatal complications including hemoptysis, pneumothorax, and cervical cellulitis occurred in nine patients. There was no mortality associated with the procedure. Despite pancytopenia, bleeding was a significant problem in only two patients.

Proposed Course:

Continue Investigation.

Publications:

Articles:

1. Aisner, J., Schimpff, S.C., Bennett, J.E., Young, V.M., Wiernik, P.H.: Aspergillus infections in patients with cancer: In association with fire-proofing materials in a new hospital. _JAMA_ 235:411-412, 1976.

2. Aisner, J., Kvols, L.K., Sickles, E.A.,Schimpff, S.C., Wiernik, P.H.: Transtracheal selective bronchial brushing for pulmonary infiltrates in patients with cancer. _Chest_ 69:367-371, 1976.

3. Aisner, J., Schimpff, S.C., Sutherland, J.C., Young, V.M., Wiernik, P.H.: Torulopsis glabrata infections in patients with cancer: Increasing incidence in relationship to colonization. Am J of Med 61:23-28, 1976.

4. Aisner, J., Schimpff, S.C., Wiernik, P.H.: Treatment of invasive Aspergillosis: Relation of early diagnosis and treatment to response. Ann Int Med 86:539-543, 1977.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 06915-04 COB

PERIOD COVERED
July 1, 1976 through Sept. 30, 1977

TITLE OF PROJECT (80 characters or less)

Staging Laparotomy and Combined Modality Therapy in Early Hodgkin's Disease

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Peter H. Wiernik, M.D.	Chief, Clinical Oncology Branch	CL NCI
	Charles H. Diggs, M.D.	Investigator	CL NCI
Others:	Robert Slawson, M.D.	Acting Director, Division of Radiation Therapy, Univ. of MD	CL NCI

COOPERATING UNITS (if any)

Radiotherapy Service and Surgery Service,
University of Maryland Hospital, Baltimore, Maryland

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline k

The aim of this study was to determine the value of staging laparotomy in Stage I and II Hodgkin's Disease. It was also to evaluate different modalities of therapy for pathologically documented Stage I and II Hodgkin's disease. Following completion of initial clinical evaluation, patients were randomized to receive laparotomy or no laparotomy if the clinical evaluation showed no evidence of Hodgkin's disease beyond Stage II. Those patients receiving no laparotomy received extended field irradiation alone and those who received laparotomy were randomized to receive limited field irradiation alone, extended field irradiation alone, or limited field irradiation plus MOPP chemotherapy. Twenty-nine patients have been randomized to date, 5 of whom have received no laparotomy and extended field irradiation, 7 with laparotomy plus involved field, 9 with laparotomy plus extended field, and 8 with laparotomy plus involved field plus MOPP chemotherapy. All patients have achieved complete remission and there have been relapses in each treatment group with the exception of the group that received adjuvant chemotherapy. The numbers of patients studied in each group are too small to make any firm evaluation of this study at this time possible. Although there is a suggestion that patients who receive adjuvant chemotherapy may do better.

1. To determine whether modification of radiation therapy ports on the basis of staging laparotomy findings will significantly improve survival of patients with clinically localized Hodgkin's disease as compared to radiation therapy administered without staging laparotomy.

2. To determine whether the addition of adjuvant chemotherapy to limited field irradiation therapy in patients with pathologically documented localized disease is beneficial.

Patients with newly diagnosed Hodgkin's disease undergo complete clinical evaluation including x-ray, hematologic, biochemical and nuclear medicine studies. If none of these studies suggests more advanced disease than Stage II, patients are randomized to receive laparotomy or no laparotomy. If no laparotomy is performed, patients receive extended field irradiation therapy. If laparotomy is performed, patients are randomized ro receive either limited field irradiation therapy, extended field irradiation therapy or limited field irradiation therapy followed by chemotherapy using MOPP.

Twenty-nine patients have been entered onto study at this point, with 5 patients receiving extended field irradiation therapy without laparotomy, 7 patients receiving extended field irradiation, and 8 patients receiving laparotomies plus involved field irradiation plus MOPP chemotherapy. All patients were able to achieve a complete remission and there have been relapses in each group except those treated with adjuvant chemotherapy. Approximately half of the relapses have occurred in patients with "E" stage disease of the lung in patients who received only irradiation therapy. Because of the limited number of patients in each treatment group, it is too early to make any firm conclusions from this study.

Publications:

1. Levi, J.A. and Wiernik, P.H.: Unfavorable prognosis and therapeutic implications of limited extranodal Hodgkin's disease. Amer J Med, (in press), 1977.

2. Levi, J.A., O'Connell, M.J., and Wiernik, P.H.: Patterns of relapse in Stage I, II, and III-A Hodgkin's disease: Influence of initial therapy and implications for the future. Blood, (in press), 1977.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 06916-04 COB

PERIOD COVERED
July 1, 1976 through September 30, 1977

TITLE OF PROJECT (80 characters or less)
Pilot Protocol for Treatment of Patients with Stage III and IV Diffuse Histiocytic Lymphoma

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Charles H. Diggs, M.D.	Investigator	CL NCI
	Peter H. Wiernik, M.D.	Chief, Clinical Oncology Branch	
Others:	Stephen C. Schimpff, M.D.	Head, Section of Infection Research	CL NCI

COOPERATING UNITS (if any)
Section of Infection Research, BCRC

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline k

This study was designed to determine the feasibility of administering high dose intensive chemotherapy for patients with advanced diffuse histiocytic lymphomas using measures to prevent infection. Patients with diffuse histiocytic lymphoma who are found to be stage III or IV after appropriate staging techniques have been placed in reverse isolation in a laminar air flow unit and have begun all non-absorbable antibiotics. They are then treated with high dose chemotherapy with cyclophosphamide 1500 mg/m^2 IV day one, adriamycin 60 mg/m^2 IV day one, vincristine 1.4 mg/m^2 IV days one and eight and a ten day course of oral prednisone at 40 mg/m^2. Eight patients have been entered to date. Myelosuppression has been the most frequently noted toxicity and though often severe has been brief. Results to date show four complete responses, one partial response and three failures. There have been three deaths of those patients achieving complete response, however. One patient died from adriamycin cardiotoxicity, one from disseminated fungemia and one patient with renal failure. The patient who has achieved a partial remission is still under therapy. Although the results of this study indicate that high dose chemotherapy with these agents is effective at achieving complete responses, further study is necessary to determine if such aggressive therapy is warranted.

Project Description: Z01 CM 06916-04 COB

This project was designed to determine whether high dose intensive combination chemotherapy of patients with diffuse histiocytic lymphomas utilizing measures to prevent infection will result in improved remission rates and prolonged survivals.

Patients with biopsy proven diffuse histiocytic lymphoma who are shown to be stage III or stage IV after staging procedures are placed in reverse isolation in a laminar air flow unit and given prophylactic non-absorbable oral antibiotics. These patients then receive therapy with cyclophosphamide 1500 mg/m^2 IV day one, adriamycin 60 mg/m^2 IV day one, vincristine 1.4 mg/m^2 IV days one and eight and a ten day course of oral prednisone 40 mg/m^2. Because cyclophosphamide and adriamycin are both myelosuppressive when given individually and since these agents are both used in full dosage here, measures to prevent infection are utilized.

Eight patients have been entered onto this study to date. Reverse isolation and the administration of oral antibiotics has been well tolerated. Myelosuppression has been severe though brief. However, one patient did die from disseminated fungal infection. There have been four complete responses noted, although three of these patients have died; one from the aforementioned fungal infection, one from adriamycin cardiotoxicity and the other from renal failure. One patient has achieved a partial response and there have been three failures. Thus, 50% of patients so far treated have achieved complete remissions at the expense of drug toxicity. Further patients are needed to more adequately evaluate this study, but at the present time infection seems to be the major factor that is limiting the success of this study.

Since disseminated diffuse histiocytic lymphoma is the most malignant variety of the non-Hodgkin's lymphomas, more aggressive chemotherapeutic regimens are needed for its treatment. If the infectious complications on this regimen can be controlled it may well represent a step forward in the treatment of this disease.

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 06917-04 COB

PERIOD COVERED
July 1, 1976 through Sept. 30, 1977

TITLE OF PROJECT (80 characters or less)

CVP versus CAVP for Disseminated Non-Hodgkin's Lymphomas

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Charles H. Diggs, M.D.	Investigator	CL NCI
Others:	Peter H. Wiernik, M.D.	Chief, Clinical Oncology Branch	CL NCI
	Charles Schiffer, M.D.	Senior Investigator	CL NCI
	Stephen Schimpff, M.D.	Senior Investigator	CL NCI

COOPERATING UNITS (if any)

None

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Clinical Oncology Branch

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TOTAL MANYEARS: 1.0	PROFESSIONAL: 1.0	OTHER: 0

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline k

Forty previously untreated patients with Stage III or IV non-Hodgkin's lymphoma were randomized to receive either CVP (C = 1.5 gm/m^2 IV day 1, V = 1.4 mg/m^2 IV days 1 and 8, P = 40 mg/m^2 PO days 1-10) or CAVP (C = 1.0 gm/m^2 IV day 1, A = 45 mg/m^2 IV day 1, V = 1.4 mg/m^2 IV days 1 and 8, P = 40 mg/m^2 PO days 1-10). All patients achieving complete remission then received CCNU 100 mg/m^2 PO day 1, bleomycin 20 mg/m^2 IM days 1 and 8, and prednisone 40 mg/m^2 PO days 1-10 (CVP) every three weeks for three courses. Eleven out of 17 (65%) CVP patients achieved CR (7/11 nodular, 4/6 diffuse) as did 15 out of 23 CAVP (9/12 nodular, 6/11diffuse). Myelosuppression was moderate with both regimens as well as with CBP treatment. Median remission durations from the end of each induction therapy will be in excess of 16 months (range 1+ to 30+) for CVP and in excess of 15 months for CAVP (range 6+ to 43+). Two out of 11 CVP patients and two out of 15 CAVP patients have relapsed to date. No patient entering CR has died of progressive lymphoma. CVP and CAVP both appear to be effective regimens for remission induction in patients with advanced non-Hodgkin's lymphomas. The addition of adriamycin as yet shows no advantages.

Patients who are found to have advanced non-Hodgkin's lymphomas, either Stage III or IV on the basis of extensive clinical and pathologic staging procedures are randomly assigned to receive therapy with either cyclophosphamide 1500 mg/m^2 IV on day 1, vincristine 1.4 mg/m^2 on days 1 and 8, and prednisone 40 mg/m^2 orally on days 1-10, or cyclophosphamide 1000 mg/m^2 IV on day 1, adriamycin 45 mg/m^2 IV on day 1, vincristine 1.4 mg/m^2 days 1 and 8 intravenously, and prednisone 40 mg/m^2 orally on days 1-10. Patients who achieve pathologically documented complete remissions then undergo consolidation therapy with CCNU, bleomycin and prednisone. A second randomization is then carried out with patients randomly assigned to receive no further therapy or to receive intermittent high dose Cytoxan monthly intravenously.

Forty patients have undergone randomization to date with 17 patients receiving CVP therapy and 23 patients receiving CAVP therapy. Eleven of 17 or 65% of the CVP patients have achieved complete remission, as have 15 of 23 patients receiving CAVP. The median duration of remission for the complete responders on CVP will be in excess of 16 months, and in excess of 15 months for CAVP. There have been two relapses in each group. No differences in the two therapy arms in remission induction or toxicities have been observed. It is too early to evaluate the effect of maintenance therapy.

Conclusions so far to be drawn from this project are: (1) the combination of Cytoxan, vincristine and prednisone is effective in inducing remission in patients with disseminated non-Hodgkin's lymphomas; (2) the addition of adriamycin to CVP confers no advantages either in remission induction or duration, and (3) diffuse histologies respond as well as nodular.

Publications:

1. Diggs, C.H., and Wiernik, P.H.: Cyclophosphamide, vincristine and prednisone (CVP) vs. cyclophosphamide, adriamycin, vincristine and prednisone (CAVP) for disseminated non-Hodgkin's lymphomas. Proc. Amer. Soc. Clin. Onc., p. 288, 1977.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 06918-04 COB

PERIOD COVERED
July 1, 1976 through Sept. 30, 1977

TITLE OF PROJECT (80 characters or less)

Streptozotocin, Bleomycin, CCNU & Adriamycin Treatment of Advanced Hodgkin's Disease

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Charles H. Diggs, M.D.	Investigator	CL NCI
Others:	Peter H. Wiernik, M.D.	Chief, Clinical Oncology Branch	CL NCI

COOPERATING UNITS (if any)

None

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☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline k

Seventeen patients with advanced previously treated Hodgkin's disease received therapy with a combination of streptozotocin 500 mg/m^2/day IV days 1-5, CCNU 100 mg/m^2 orally day 1, adriamycin 45 mg/m^2 IV day 1, and bleomycin 15 mg/m^2 IM days 1 and 8 at 28 day intervals. The overall response rate was 59% with 6 patients (35%) achieving complete remissions and 4 patients (24%) entering partial remissions. Median duration of complete remission is 8+ months with a range of 2+ to 18+ months. The median duration of survival from the start of therapy for the complete responders was 16+ months with a range of 5+ to 25+ months. Myelosuppression occurred regularly and was severe in 25% of courses. Other toxicities included bleomycin pulmonary toxicity in three patients, renal tubular dysfunction secondary to streptozotocin in 3 patients and hepatic dysfunction in 3 patients. SCAB has been shown to be an active combination of agents which is not cross-resistant to MOPP-type regimens.

To determine clinical usefulness of new combination chemotherapy for advanced Hodgkin's disease in patients no longer responsive to conventional chemotherapies.

Patients with advanced biopsy-proven Hodgkin's disease who are no longer responsive to other agents receive streptozotocin 0.5 gm/m^2 daily for 5 days intravenously, CCNU 100 mg/m^2 PO day 1, bleomycin 15 mg/m^2 IM days 1 and 8, and adriamycin 45 mg/m^2 IV day 1. Cycles are repeated each 28 days.

Seventeen patients have been treated with this regimen. Six patients (or 35%) have achieved a complete remission, and an additional 4 patients (or 24%) have entered partial remissions, for an overall response rate of 59%. This combination of agents appears to have a definite activity in this previously-treated group of patients with Hodgkin's disease.

MOPP chemotherapy for advanced Hodgkin's disease has become considered as standard for these patients. Between 60 and 80% of patients treated with MOPP will achieve complete remission, yet there remains a sizable percentage of these patients who do not achieve complete remission. Therefore, the development of effective alternative regimens is necessary either for achieving complete remissions in greater percentages of patients or for treating patients who have not responded to MOPP. Streptozotocin, CCNU, adriamycin and bleomycin all have definite activity against Hodgkin's disease, and since each has different toxicities the combination of the four drugs would seem feasible. These agents as used in this combination have shown activity in patients with previously-treated Hodgkin's disease, although myelosuppression was perhaps more of a problem than with MOPP. A contributing factor, however, would be that all these patients had received prior chemotherapy, making their marrows more sensitive to chemotherapeutic agents. Because of the activity of this regimen, it is now being tried in previously untreated patients with advanced Hodgkin's disease.

Publications:

1. Levi, J.A., Wiernik, P.H., and Diggs, C.H.: Combination chemotherapy of advanced previously-treated Hodgkin's disease with streptozotocin, CCNU, adriamycin and bleomycin. Medical and Pediatric Oncology 3, pp. 33-40, 1977.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 06919-03 COB

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Initial Drug Therapy for Metastatic Renal Cell Carcinoma

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	D. M. Hahn, M.D.	Senior Investigator	CL NCI
Others:	S. C. Schimpff, M.D.	Head, Section of Infection Research	CL NCI
	P. H. Wiernik, M.D.	Chief, Clinical Oncology Branch	CL NCI

COOPERATING UNITS (if any)

None

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline k

Because renal cell carcinoma is notoriously resistant to chemotherapy and radiation, and only occasionally cured by surgery, a prospective randomized controlled chemotherapy trial was established to compare efficacy of PO CCNU, IV vinblastine, and IV Thiotepa. Patients failing to respond to first line therapy were then treated with IM bleomycin. 26 patients with metastatic renal cell carcinoma have been randomized between the three first line forms of therapy, and 7 patients had received bleomycin. The three method treatment groups are equivalent in age, prior therapy, time from diagnosis to therapy and incidences of CNS metastases. Nine patients were treated with CCNU and 7 were evaluable; there were no responses, 1 stabilization and 6 progressions of the disease. Ten patients received vinblastine with 2 objective responses; one stabilization and 7 progressions. Seven patients received Thiotepa and there was 1 month partial remission, 1 stabilization and 5 progressions. Of the 7 patients treated with bleomycin there were 2 stabilizations and 5 progressions. Mild-to-moderate myelosuppression was a major side effect for each drug regimen. Emesis occurred in only the CCNU group. Survival times were equivalent for all three primary forms of therapy and did not differ from historical controls. The protocol has been discontinued and a paper is being submitted for publication.

Objectives:

Renal carcinoma is a solid tumor of notorious resistance to chemotherapy and radiation. Surgery may be curative in some cases, but the overwhelming percentage of cases proceed to metastatic disease and death within two years of diagnosis. Most previous chemotherapy studies have been inadequate. Several previous well-controlled studies indicated that adriamycin had no activity and provera had only minimal activity. Drugs that have shown some efficacy in preliminary trials included CCNU with a 10 to 15% response rate, vinblastine with approximately the same response rate and bleomycin which had less of a response rate. In addition, a drug derivative of Thiotepa which had been tried in the Soviet Union, was very promising with approximately 50% response rate. Therefore, a study was set up to randomize between vinblastine, CCNU and Thiotepa for the first line of therapy and if the patient relapsed from this therapy then a second round of therapy would be bleomycin unless there was sufficient reason not to give the bleomycin because of chronic obstructive pulmonary disease.

Major Findings:

Twenty-six patients with metastatic hypernephroma have been randomized between the three first line forms of therapy. Three groups are equivalent in age, prior therapy, and time from diagnosis to therapy and incidence of CNS metastases. Nine patients have received CCNU therapy and 7 of these are evaluable. This therapy resulted in no partial responses, 2 stabilizations and 5 progressions. Vinblastine has been received by 10 patients. There have been 2 objective responses, 1 stabilization and 7 progressions. Thiotepa has been received by 7 patients. There have been 1 short-lived partial response, 1 stabilization and 5 progressions. Seven patients have received bleomycin and 2 have had stabilization, but one has had progressive disease. Myelosuppression was the major side effect in each of the initial drug therapies with no complications other than therapy delayed resulting from this. Emesis occurred in the CCNU group (9 patients). Survival time was equivalent in all 3 groups and did not differ from historical controls. Brain metastasis was associated with early death in 3 out of 4 patients and a lack of response to therapy in all patients. Thus, minimal activity was demonstrated for Thiotepa and vinblastine and no responses were seen in CCNU or bleomycin.

Publications: Pending.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 06921-04 COB

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)
Adriamycin Therapy for Advanced Mycosis Fungoides

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Peter H. Wiernik, M.D.	Chief, Clinical Oncology Branch	CL NCI
	Charles H. Diggs, M.D.	Investigator	CL NCI

COOPERATING UNITS (if any)
None

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline k

This study has been designed to determine the efficacy of adriamycin therapy for the treatment of advanced mycosis fungoides. Adriamycin has been administered to patients with biopsy proven diagnosis of mycosis fungoides who were refractory to any prior therapy. Disease status was advanced in all patients with extensive skin involvement and lymphadenopathy. Visceral disease was frequent. Adriamycin was administered in a dose of 60 mg/m^2 IV every three weeks and a minimum of three courses was given beyond complete response. Maintenance therapy consisted of methotrexate 15 mg/m^2 IM twice weekly and Cytoxan 750 mg/m^2 IV every three weeks. Thirteen patients were entered onto this study. There have been three complete responses, five partial responses and three improvements, with two failures. Toxicity has been mild and it would appear that adriamycin is an active agent for the treatment of advanced mycosis fungoides.

PHS-6040
Rev. 10-76

This project was designed to determine the efficacy of adriamycin therapy for the treatment of advanced mycosis fungoides.

Adriamycin was given to patients with biopsy proven diagnosis of mycosis fungoides and who had received previous therapy but now were refractory. Lymphadenopathy was present in all cases and all patients had extensive skin disease. Adriamycin was administered in a dose of 60 mg/m^2 IV every three weeks with dosage modification for hepatic dysfunction or if significant myelosuppression occurred. A minimum of three courses were given and if a complete response occurred, a further three courses beyond that point were given. Maintenance therapy was administered with methotrexate 15 mg/m^2 IM twice weekly and Cytoxan 750 mg/m^2 IV every three weeks. Of the 13 patients entered onto study, there have been three complete responses (23%), five partial responses (39%), three improvements (23%) and two failures. Toxicity was mild with only a moderate degree of myelosuppression noted. It would appear, therefore, that adriamycin is an effective agent for the treatment of advanced mycosis fungoides.

Once mycosis fungoides reaches an advanced stage there is often rapid progression to eventual death. If single agents can be shown to be effective in this disease, perhaps more effective combinations of agents can be used to prolong survival time. Adriamycin would appear to be one of these agents. This project will now be closed.

Publications:

1. Levi JA, Diggs CH, and Wiernik PH: Adriamycin therapy for advanced mycosis fungoides. Cancer 39, 1977.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 06939-02 COB

PERIOD COVERED

July 1, 1976 through September 30, 1977

TITLE OF PROJECT (80 characters or less)

Methotrexate, Oncovin, L'Asparaginase and Dexamethasone Therapy for Adults with ALL

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Robert J. Esterhay, Jr., M.D.	Senior Investigator	CL NCI
	Peter H. Wiernik, M.D.	Chief, Clinical Oncology Branch	CL NCI

COOPERATING UNITS (if any)

None

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2	1.5	0.5

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☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline k

The purpose of this project is to improve the remission induction rate of acute lymphocytic leukemia in adult patients utilizing vincristine and dexamethasone with intermittent moderate doses of methotrexate followed by L'asparaginase. A second objective is to decrease the incidence of severe infections during induction therapy by utilizing oral non-absorbable antibiotics. A third objective is to determine whether intermittent high dose methotrexate with leucovorin rescue and with vincristine and dexamethasone will prolong remission duration. The fourth objective is to determine the efficacy of moderate and high dose methotrexate in preventing meningeal leukemia and to correlate the clinical effectiveness with the cerebrospinal fluid methotrexate levels. To date 13 patients have been entered onto this study. Seven patients had no prior treatment, six had prior treatment. For the prior treatment patients, one is too early to evaluate, six have been through or are currently on induction, two are on consolidation, one cytoreduction and one is out to maintenance. For the no prior treatment patients, one is too early to evaluate, five have been through or are currently on induction, two are on consolidation, one cytoreduction and one is out to maintenance. For the no prior treatment patients, complete remissions have been achieved in five of six patients that went through induction. For the prior treatment patients, three achieved complete remission and three died.

PHS-6040
(Rev. 10-76)

The purpose of this project is to improve the remission induction rate of Acute Lymphocytic Leukemia (ALL) in adult patients utilizing vincristine and dexamethasone with intermittent moderate doses of methotrexate followed by L'asparaginase. A second objective is to decrease the incidence of severe infections during induction therapy by utilizing oral non-absorbable antibiotics. The third objective is to determine whether intermittent high dose methotrexate with leucovorin rescue and with vincristine and dexamethasone will prolong remission duration in a significant percentage of adult patients with ALL. The fourth objective is to determine the efficacy of moderate and high dose methotrexate in preventing meningeal leukemia and to correlate clinical effectiveness with the cerebrospinal fluid methotrexate levels.

Patients 15 years of age and over with a diagnosis of ALL will be eligible. Induction therapy will consist of methotrexate 100 mg/m^2 IV push on day one only, dexamethasone 6 mg/m^2 orally daily, vincristine 2 mg (regardless of body surface area) IV push on day two only, and L'asparaginase 500 IU/kg IV infusion over a 30 minute period on day two only. The induction therapy is to be continuous with the second course beginning on day eleven, the third course on day 21, etc. Patients achieving a complete remission precede to consolidation therapy which consists of continuing with only methotrexate and L'asparaginase. The consolidation therapy consists of six cyclic courses which begins the day after the completion of induction therapy. Patients remaining in complete remission after six courses of consolidation therapy will proceed to cytoreduction therapy, which consists of vincristine, dexamethasone, high dose methotrexate (100 mg/kg) followed by citrovorum factor (5% of the high dose methotrexate dose (mg/mg) over three days). Cytoreduction therapy is given monthly for a total of 12 courses. Maintenance therapy begins after the completion of cytoreduction and is continued indefinitely until bone marrow relapse with each course given at four week intervals. Maintenance therapy consists of vincristine on day one, dexamethasone orally on days one through five, methotrexate (15 mg/m^2) orally weekly, and 6-mercaptopurine orally daily.

A total of 13 patients have been entered onto this study. Seven patients had no prior treatment, six had prior treatment. For the prior treatment patients, one is too early to evaluate, six have been through or are currently on induction, two are on consolidation, one cytoreduction and none are out to maintenance. For the no prior treatment patients, one is too early to evaluate, five have been through or are currently on induction, two are on consolidation, one cytoreduction and one is out to maintenance. For the no prior treatment patients, complete remissions have been achieved in five of six patients that went through induction. The one patient that died from infection had a definite clinical improvement by bone marrow exam and at autopsy had no evidence of lymphoblasts. For the prior treatment patients, three have achieved complete remission and three have died. The major toxicity with the program is related to the E. coli strain of L'asparaginase. Every patient has had to be switched to the Erwinia strain. The 100% response rate is only true now for the previously untreated patients; nevertheless, the results continue to be very encouraging.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 06942-02 COB

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Development of the Acoustic Microscope as a Useful Tool in Hematology

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Peter H. Wiernik, M.D.	Chief, Clinical Oncology Branch	CL NCI
Other:	C. F. Quate, Ph.D.	Professor of Applied Physics & Electrical Engineering	Stanford Univ. Stanford, Ca.

COOPERATING UNITS (if any)
Stanford University, Stanford, California

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline k

The acoustic microscope, which utilizes microwave sound rather than light or electron beams to reproduce an image, is investigated in this study for its possible usefulness in hematologic research. Early results indicate that appropriate resolution is possible.

PHS-6040
(Rev. 10-76)

The acoustic microscope, developed at Stanford University, utilizes microwave sound rather than light or electrons to reproduce the image of a microscopic object. Objects to be examined by the instrument need no fixing or staining. We are endeavoring to adapt this instrument for the examination of peripheral blood and bone marrow by addressing ourselves to problems of resolution. Early results are encouraging and indicate that this instrument may ultimately be useful for rapid examination of hematologic specimens.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 06944-02 COB

PERIOD COVERED
July 1, 1976 through Sept. 30, 1977

TITLE OF PROJECT (80 characters or less)

Treatment of Oat Cell Carcinoma of the Lung

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Joseph Aisner, M.D.	Senior Investigator	CL NCI
Others:	Robert J. Esterhay, Jr., M.D.	Senior Investigator	CL NCI
	Peter H. Wiernik, M.D.	Chief, Clinical Oncology Branch	CL NCI

COOPERATING UNITS (if any)

None

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline k

Oat cell carcinoma of the lung is a rapidly fatal disease. Regardless of therapy, nearly all patients die of metastases, suggesting that this tumor is a disseminated disease. This study evaluates combination chemotherapy with 3 active agents (cyclophosphamide, adriamycin, and VP 16-213) and immunotherapy with MER for its potential to increase response rate duration, or survival as well as its potential to alter drug toxicity. 34 patients have been entered. There have been 14 complete remissions, 10 partial remissions, 4 lesser responses, 3 patients without measurable disease, and 3 patients without response. 28 patients had received no prior therapy. 2 of these patients had no measurable disease, and the remaining 26 showed evidence of response. 24 of these 26 had either complete or partial response. 5 of 10 patients with partial response are still improving. Among 6 patients previously irradiated, 3 failed to respond, 2 died from hematologic toxicity, and 1 has no measurable disease. Whether the patient had limited or widespread disease had no appreciable effect on response rate or durations. MER has had no appreciable effect on response rate, duration, survival or drug toxicity. Toxicity was moderately severe including alopecia, nausea and vomiting and blood count suppression. Toxicities were manageable by dose modification. There were 3 drug-related deaths, and 2 patients died of arrhythmias. MER toxicity included fever, chills, pain, local inflammation and weeping ulcers.

Since nearly all patients with small cell carcinoma of the lung (oat cell) die of metastases, this disease can be considered disseminated at the time of presentation. Despite most forms of localized therapy, patients have a short survival and aggressive chemotherapy is thus warranted. Cyclophosphamide, adriamycin, and VP 16-213 have all been shown to be active single agents in the therapy of this disease. In addition, cyclophosphamide and adriamycin have been shown to have synergistic antineoplastic activity in animal models. Since the duration of response to all forms of therapy in the past has been relatively short, MER immunotherapy is being evaluated for its potential to increase response rate, duration, survival or to alter potential drug toxicity or to augment drug effects. A randomization for the use of MER immunotherapy is used to test these latter concepts. As of April 1977, 34 patients have been entered onto protocol. Overall there were 14 complete responses, 10 partial responses with 5 of 10 still improving, 4 objective responses (less than a partial response), 3 patients without measurable disease, all 3 surviving one year beyond diagnosis, and 3 patients who failed to respond. Patients were stratified according to prior radiotherapy or no previous treatment. Six patients received prior radiotherapy, 1 patient is without measurable disease (surviving greater than 1 year), 2 patients had objective tumor regression but died of hematologic toxicity, and 3 patients failed to respond. The median duration of survival from day of diagnosis for these 6 patients was 135 days. 28 patients received no prior therapy. 2 patients are without measurable disease, surviving greater than one year. All the remaining 26 patients had at least an objective response, and 24 of these 26 patients had either complete or partial remissions. Median duration of survival for previously nontreated patients has not been reached and is in excess of 222 days. Response and survival durations are longer for complete responders than for partial responders. Patients were staged as limited or widespread disease. Eleven patients had limited disease and 23 had widespread disease. Whether the patient had limited or widespread disease had no appreciable effect on the various response rates, response durations or survivals. MER has had no significant effect on response rate, response duration, or survival. MER has also shown no effect on drug toxicity. Toxicity was moderately severe and included alopecia, nausea and vomiting, and blood count suppression. Toxicities were generally manageable by dose modification. Nine patients had serious infectious episodes during periods of neutropenia and 3 such patients died from these infections. All 3 patients were responding to therapy. In addition, 2 patients with improving partial responses died of myocardial infarction from pre-existing cardiovascular disease. There were no other drug-related deaths and most patients received all therapy as outpatients. MER toxicity included fever, chills, local pain and weeping ulcer formation. MER-induced fever during periods of neutropenia resulted in 8 hospitalizations among 5 patients.

Proposed Course:

Continue investigation.

Publications:

Articles:

1. Aisner, J., Wiernik, P.H., Esterhay, R.J. jr: Treatment of small cell carcinoma of the lung with cyclophosphamide, adriamycin and VP 16-213 with or without MER. in Adjuvant Therapy of Cancer, Salmon, S.E. and Jones, S.E. (Eds), Elselvier/North Holland Press, 1977 (In press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 06947-01 COB

PERIOD COVERED
July 1, 1976 through September 30, 1977

TITLE OF PROJECT (80 characters or less)

Amphotericin-B and Adriamycin Combination Therapy of Refractory Neoplasia

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Paul Chang, M.D.	Senior Investigator	CL NCI
	Peter H. Wiernik, M.D.	Chief, Clinical Oncology Branch	CL NCI
Others:	Nicholas Bachus, M.D. Ph.D.	Chief, Biochemistry Branch	LPH NCI

COOPERATING UNITS (if any)

None

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0.5	0.5	0

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☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline ke

In vitro and in vivo laboratory studies indicate that amphotericin enhances the cell uptake and cytotoxicity of many compounds, including some of the antitumor antibiotics. This study is an attempt to determine if there is a clinically recognizable enhancement of the therapeutic index of adriamycin when combined with amphotericin.

PHS-6040
(Rev. 10-76)

Amphotericin-B, a polyene, antifungal, antibiotic, is able to augment the cellular uptake of many compounds, including certain antitumor antibiotics. Other laboratories have demonstrated an enhanced therapeutic index both *in vitro* and *in vivo* for BCNU with amphotericin. Other experiments with tumor cells resistant to actinomycin-D because of decreased cellular uptake of the drug, demonstrate an augmented cellular uptake and enhanced cytotoxicity of actinomycin-D when these drug resistant cells were exposed to amphotericin with actinomycin-D. Studies in our own laboratory with L1210 leukemia have demonstrated impressive enhancement of survival of mice receiving both amphotericin and the anthracycline antibiotic, daunorubicin, compared with leukemic mice receiving either drug alone.

Recent clinical results have been reported with a combination of adriamycin, BCNU, cyclophosphamide and amphotericin. Responses were obtained in patients with acute non-lymphocytic leukemia, carcinoma of the breast and myeloma already resistant to the combination of adriamycin, BCNU and cyclophosphamide alone. Although fever and chills accompanied the administration of amphotericin, myelosuppression was not felt to be uniformly increased.

This study, which has just been started and has no reportable results, will use amphotericin combined with adriamycin in patients who are already resistant to adriamycin, to see if there is any augmentation of the therapeutic index of adriamycin.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 06948-01 COB

PERIOD COVERED
July 1, 1976 through September 30, 1977

TITLE OF PROJECT (80 characters or less)
Treatment of Chronic Myelocytic Leukemia

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Robert Esterhay, M.D.	Senior Investigator	CL NCI
	Peter H. Wiernik, M.D.	Chief, Clinical Oncology Branch	CL NCI

COOPERATING UNITS (if any)
None

LAB/BRANCH
Clinical Oncology Branch

SECTION
Office of the Chief

INSTITUTE AND LOCATION
NCI, NIH   Baltimore, Maryland 21201

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1	1	0

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS   ☐ (b) HUMAN TISSUES   ☐ (c) NEITHER

☐ (a1) MINORS   ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline k

The first study for the treatment of chronic myelocytic leukemia is designed to determine whether or not more intensive treatment directed against the presumed abnormal myeloblasts can postpone or prevent myeloblastic transformation. The second study is designed to assess the effectiveness of anthracycline antineoplastic therapy in the induction treatment of the transformation phase of chronic myelocytic leukemia once it does develop. The objective of the first study is to determine whether CCNU and Ara-C begun at the time of diagnosis of CML without busulfan can postpone or prevent myeloblastic transformation while the objectives of the second study are to assess the effectiveness of the addition of daunorubicin to hydroxyurea, 6-mercaptopurine and prednisone as induction treatment of the blastic or "resistant" phase of CML. In the first study, patients are randomized to control treatment with busulfan followed by continuous "maintenance" busulfan. CCNU and Ara-C given every six weeks starting at the time of entry into the study. In the second study patients are randomly assigned to one of two treatment programs designed to compare the relative effectiveness of daunorubicin, hydroxyurea, 6-mercaptopurine and prednisone with or without the addition of MER immunotherapy. As of June, 1977, 12 patients have been entered onto these two studies. It is too early to evaluate response and survival rates.

None of the traditional methods of treatment have significantly increased the survival of patients with chronic myelocytic leukemia (CML). The median duration of survival of patients with CML is still approximately 3-3.5 years, about the same as that described in 1924 for both treated and untreated patients. Myeloblastic transformation is the main cause of death; approximately 70% of patients die in this stage of the disease. Since there is no striking effective treatment for this phase of the disease once developed, a reasonable approach to prolong survival is to prevent the onset of the blast crisis. The first study is designed to determine whether or not more intensive treatment directed against the presumed abnormal myeloblast can postpone or prevent myeloblastic transformation. The second study is designed to assess the effectiveness of daunorubicin in the induction treatment of the blastic phase of CML once it does develop.

The objective of the first study is designed to determine whether CCNU and Ara-C begun at the time of diagnosis of CML without busulfan can postpone or prevent myeloblastic transformation. The objectives of the second study are as follows: to assess the effectiveness of the addition of daunorubicin to hydroxyurea, 6-mercaptopurine, and prednisone as induction treatment of the blastic or "resistant" phase of CML; to evaluate whether intermittent intravenous cyclophosphamide, added to maintenance therapy with hydroxyurea, 6-mercaptopurine and prednisone can prolong the duration of remission when compared to historical controls; to determine whether early immunotherapy with MER will increase the probability of achieving complete remission and prolonging its duration.

In the first study, patients with CML are randomized to "control" treatment consisting of induction of remission with busulfan followed by continuous "maintenance" busulfan to maintain the WBC between 4,000 and 15,000/mm^3 or to CCNU and Ara-C every 6 weeks starting at the time of entry into the study. In the second study patients with the blastic or "resistant" phase of CML are randomly assigned to one of two treatment programs designed to compare the relative effectiveness of daunorubicin, hydroxyurea, 6-mercaptopurine and prednisone with or without the addition of MER immunotherapy to prior experience with the hydroxyurea, 6-mercaptopurine, prednisone combination. All patients with "resistant" CML who achieve complete or partial remission status after induction treatment will receive the same maintenance treatment with daily hydroxyurea, 6-mercaptopurine, pulsed prednisone and daunorubicin to the maximum allowable cumulative dose.

As of June, 1977, 12 patients have been entered onto these two studies.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 06949-01 COB

PERIOD COVERED
July 1, 1976 through Sept. 30, 1977

TITLE OF PROJECT (80 characters or less)

Combination Chemotherapy for Metastatic Carcinoma of the Colon

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Charles H. Diggs, M.D.	Investigator	CL NCI
	Peter H. Wiernik, M.D.	Chief, Clinical Oncology Branch	CL NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH
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SECTION
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INSTITUTE AND LOCATION
NCI, NIH Baltimore, MD 21201

TOTAL MANYEARS: 0.5	PROFESSIONAL: 0.5	OTHER: 0

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline ke

Twenty-one patients with advanced metastatic adenocarcinoma of the colon or rectum were treated with a combination of intravenous Methyl CCNU and Ftorafur, an analogue of 5-fluorouracil. In addition, 13 patients were randomized to receive MER. Two previously untreated patients, neither of whom had received MER, achieved partial remissions lasting 9+ and 31 weeks. Neither patient had hepatic involvement with disease. Four patients had stabilization of disease lasting up to 14 weeks. Three of these patients had received MER. Hematologic toxicity of this regimen was severe with 65% of all patients treated having platelet counts below 100,000/mm^3 at some time during the course of therapy. 33% of patients had white blood cell counts of 2,000/mm^3 at some time during therapy. Although others have reported optimistic results from similar drug combinations, the results of this study are not encouraging. It is postulated that hepatic conversion of ftorafur to 5-FU may occur less well in patients with hepatic dysfunction. Further studies of ftorafur metabolism in humans are needed.

1. To assess the value of the combination of intravenous Methyl CCNU and ftorafur in patients with metastatic adenocarcinoma of the colon or rectum.

2. To assess the value of immunotherapy in addition to combination chemotherapy.

Adenocarcinoma of the large bowel is a disease which has been consistently resistant to all forms of nonsurgical therapy. Although 5-fluorouracil remains the most commonly used medication for this disease, combinations of chemotherapeutic agents have been shown to be superior to 5-FU as a single agent. The combination of Methyl CCNU and 5-FU has given high response rates, and this study was designed to assess whether the intravenous form of Methyl CCNU combined with ftorafur with or without immunotherapy was beneficial.

Twenty-one patients were treated on this regimen, all of whom had objectively measurable sites of metastatic disease. There were 16 males and 5 females with a median age of 56 years. Eighteen of these patients were fully ambulatory at the start of therapy. Only one patient had received prior chemotherapy and 4 patients had received local radiation therapy. Thirteen patients had hepatic involvement with metastatic disease either alone or in combination with other sites of metastasis. Of the 21 patients treated, only two patients (or 9%) were able to achieve partial remissions of their disease. Neither of these patients had hepatic involvement with metastatic disease. One patient had a non-surgically-resectable abdominal mass, the other patient having a left neck mass. Severe hematologic toxicity was noted with this regimen, with 65% of all patients having platelet counts below 100,000/mm^3 at some time during their therapy.

Possible reasons for the lack of a high percentage of responders may be that intravenous Methyl CCNU is not as active a drug as oral Methyl CCNU. The hematologic toxicity with thrombocytopenia being severe would seem to indicate, however, that the drug was active. Ftorafur, on the other hand, must be metabolized to 5-fluorouracil for it to become active. Since animal studies have suggested that hepatic tissue is a site in which ftorafur is metabolized to 5-FU, it is possible that patients with metastatic disease in their livers do not metabolize ftorafur to its active form. Immunotherapy with MER, likewise, did not seem to increase the response rate.

Because of the lack of efficacy of this treatment regimen, this project is closed to further patient accrual.

Publications:

1. Diggs, C.H., Wiernik, P.H., Smythe, A.C., and Sutherland, J.C.: Intravenous Methyl-CCNU and ftorafur ± MER for metastatic colorectal carcinoma. Proc Amer Assoc for Cancer Res, p 134, May, 1977.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 06952-01 COB

PERIOD COVERED
July 1, 1976 through Sept. 30, 1977

TITLE OF PROJECT (80 characters or less)

Mycosis Fungoides Cooperative Study Group

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Charles H. Diggs, M.D.	Investigator	CL NCI
	Peter H. Wiernik, M.D.	Chief, Clinical Ocology Branch	CL NCI
Others:	Stanford I. Lamberg, M.D.	Chairman, Steering Committee, Baltimore City Hospital, Baltimore, MD	

COOPERATING UNITS (if any)

All institutions in the Cooperative Study Group.

LAB/BRANCH
Clinical Oncology Branch

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INSTITUTE AND LOCATION
NCI, NIH Baltimore, MD 21201

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.25	0.25	0

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☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline k

The Mycosis Fungoides Cooperative Group was founded in order to study both the natural history of and various treatment regimens for mycosis fungoides in all stages of the disease. To date, 400 cases of pathologically documented mycosis fungoides have been accrued and follow-up information is current on more than 95% of these patients. All data have been computerized and are in the process of being examined to seek correlations with various demographic, history and physical examination, laboratory and pathologic data. Forty patients to date have been randomized on the chemotherapy arm which attempts to study both chemotherapy, radiotherapy and topical therapy for the disease. Of these 40 patients, 3 have been entered from the Baltimore Cancer Research Center onto treatment arms. One patient failed therapy, one patient died from bleomycin pulmonary toxicity, and one patient is presently being followed. Of the 400 study patients who are being followed but not on chemotherapy protocols from the study group, there have been 14 from the BCRC. All patients continue to be followed by the BCRC. There are no firm conclusions yet from this study.

PHS-6040
(Rev. 10-76)

Mycosis fungoides is frequently an indolent skin disease that presents many years before any diagnostic pattern appears on pathologic specimens. It is the intent of this study group to assess what the course of mycosis fungoides is from the time of pathologic documentation as well as what the course of pre-mycosis fungoides lesions may be. It is also the intent to study chemotherapy in patients with advanced cutaneous disease and in patients with visceral mycosis fungoides.

Patients who have received previous therapy for mycosis fungoides are presently being followed on no protocol therapy by the Cooperative Group in order to determine both the natural history of the disease and to accumulate more information concerning these patients. Patients who have received no prior therapy are being randomized to one of several treatment regimens. Patients with poor prognosis cutaneous disease without visceral mycosis fungoides, that is, those patients who have plaque disease over greater than 10% of their body surface area and palpable lymphadenopathy, or those patient with multiple tumors with or without lymphadenopathy, are being randomized to receive electron beams, radiotherapy or topical nitrogen mustard therapy. In addition, some patients are being randomized to receive no adjuvant systemic therapy, single-dose methotrexate therapy at 500 mg/m^2 followed by citrovorum rescue every 3 weeks, with dosage increments on each successive course, or to low-dose CHOP chemotherapy with cyclophosphamide 500 mg/m^2 IV, adriamycin 40 mg/m^2 IV, and vincristine 1.4 mg/m^2 IV plus prednisone 60 mg/m^2 orally days 1-5 repeated every 21 days. Those patients with visceral mycosis fungoides are allocated to one of three treatment regimens. Regimen 1 consists of methotrexate 500 mg/m^2 with dosage increments as described above, with citrovorum rescue alone. Regimens 2 and 3 consist of methotrexate with citrovorum rescue, adriamycin, bleomycin and azarabine, either given concurrently or given on successive weeks.

Three patients have been randomized from the BCRC to receive chemotherapy. One patient failed low-dose CHOP, one patient experienced fatal bleomycin pulmonary toxicty, the other patient is still being treated. There are an additional 11 patients who are being followed on therapy at the BCRC, having been entered only in the demographic part of this study.

Four hundred cases have been accrued for follow-up study, and 40 patients have been randomized to receive one of the therapy arms. These patients are now being analyzed by the headquarters office and the results should be available soon. Patients will still be accrued on study.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 06953 01 COB

PERIOD COVERED
July 1, 1976 through Sept. 30, 1977

TITLE OF PROJECT (80 characters or less)

Chemotherapy for Metastatic Carcinoma of the Breast

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Joseph Aisner, M.D.	Senior Investigator	CL NCI
Others:	Peter H. Wiernik, M.D.	Chief, Clinical Oncology Branch	CL NCI

COOPERATING UNITS (if any)

Cancer and Leukemia Group B

LAB/BRANCH
Clinical Oncology Branch

SECTION
Office of the Chief

INSTITUTE AND LOCATION
NIH, NCI Baltimore, Maryland 21201

TOTAL MANYEARS: 1/2	PROFESSIONAL: 1/2	OTHER: 0

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☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline ke

Combination chemotherapy for metastatic carcinoma of the breast utilizing combinations of active single agents can produce high response rates with improvement in the patients' survival. There are many active combinations with response rates which vary from 40-80%. There is a wide variation in reported response rates, but there are few prospective comparative trials of the various treatment regimens, and few studies which stratify for the prognostic variables. The current study randomly compares 3 active combination chemotherapy regimens for response rates, response duration, and survival. In addition, the current study evaluates the effect of MER immunotherapy upon response rate, duration, survival, or its effect on drug toxicities. Since carcinoma of the breast if a very heterogenous disease and there are multiple treatment arms in this protocol, a large cooperative group effort is necessary to stratify for all the variables and treatment arms. The protocol was activated October 11, 1976, and 102 patients are currently on protocol. However, it is too early for the evaluation of any data.

PHS-6040
(Rev. 10-76)

Since the introduction of the 5-drug combination chemotherapy program for advanced breast cancer, there have been a large number of studies which have used various permutations of the drugs and their scheduling. In addition, the new anthracycline antibiotic, adriamycin, has been used in several regimens in an attempt to improve response rate ahd duration of response. All of these studies have clearly shown that combination chemotherapy has improved the response rate, duration and survival among patients with metastatic disease when compared to the use of single agents. However, few of these studies prospectively compare the various treatment regimens that have been developed. Metastatic carcinoma of the breast is a very heterogenous disease with multiple factors which influence the course of metastatic disease. Thus, in order to do a prospective comparative trial of several different chemotherapy programs, a large group effort is necessary to stratify for these prognostic factors. The current study randomly compares 3 combination chemotherapy regimens, each of which has been shown to be an effective treatment program for metastatic breast cancer. In addition, the current study evaluates the role of MER immunotherapy for its potential to increase response rate, response duration, survival, or its effect on drug toxicity. The role of MER immunotherapy is also being evaluated in a prospective randomized manner. All treatment programs are clearly stratified for the recognized prognostic factors.

As of April 1977, 102 patients have been entered onto the protocol from multiple institutions. These patients have been roughly equally distributed among the various treatment groups. All interval analyses of data will be encoded to avoid recognition and avoid investigative bias. At the present time, no encoded data are available for analysis.

Proposed Course:

Continue Investigation.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 069[illegible]4-01 COB

PERIOD COVERED
July 2, 1976 through Sept. 30, 1977

TITLE OF PROJECT (80 characters or less)

Chemotherapy for Carcinoma of the Head and Neck

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Peter H. Wiernik, M.D.	Chief, Clinical Oncology Branch	CL NCI
	E. George Elias, M.D., Ph.D.	Professor of Surgery, University of Maryland Hospital	CL NCI
Others:	Joseph Aisner, M.D.	Senior Investigator	CL NCI

COOPERATING UNITS (if any)

Department of Surgery, University of Maryland Hospital

LAB/BRANCH
Clinical Oncology Branch

SECTION
Office of the Chief

INSTITUTE AND LOCATION
NIH, NCI Baltimore, Maryland 21201

TOTAL MANYEARS: 1/2	PROFESSIONAL: 1/2	OTHER: 0

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☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline ke

Advanced squamous cell carcinoma of the head and neck or esophagus have very poor five-year survivals despite aggressive local treatment with surgery, radiotherapy or both. Many of such patients die of metastatic disease. Therefore, pilot trial of aggressive combination chemotherapy is warranted to determine if improved response rate or survival can be obtained. Bleomycin, high-dose methotrexate with citrovorum rescue, and cis-platinum-2 are single agents with activity in these tumors. The current study utilizes a combination of these 3 agents to determine toxicity, response rate, and response duration. Seven patients have been entered onto this pilot study and 4 are too early to evaluate. Two patients have achieved a partial response and 1 patient has shown objective regression of tumor. The patients who have had objective regression of tumor may now be technically resectable. Toxicities have been manageable by careful hydration, and careful folinic acid reversal.

Advanced squamous cell carcinoma of the head and neck and esophagus have a very poor survival rate despite aggressive surgery, radiotherapy or both. Extensive surgery or radiotherapy for such diseases may lead to inanition. Many of such patients develop metastases despite control of the local tumor. Several single agents have been identified with activity in these tumors, and combination chemotherapy may thus be warranted. The current pilot study utilizes a combination of 3 active agents: bleomycin, high-dose methotrexate with folinic acid rescue, and cis-diaminodichloride platinum,. to test the toxicity of such combination program, and to determine if such a combination therapy increases response rate, duration, makes the tumors more technically resectable, and ultimately improves survival.

As of April 1977, seven patients have been entered onto the study. Four patients are too early to evaluate. Two patients have achieved a partial response and have technically more easily resectable tumors. One patient has had an objective tumor regression and continues to respond. There have been no significant drug toxicities with careful alkalinization and hydration as well as careful detail to the folinic acid reversal. All patients receive at least one course of therapy and those patients with evidence of response will receive further chemotherapy.

Proposed Course:

Continue investigation.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 06955-01 COB

PERIOD COVERED
July 1, 1976 through September 30, 1977

TITLE OF PROJECT (80 characters or less)

Adjuvant Chemotherapy and Combination Chemoimmunotherapy in the Treatment of Advanced Malignant Melanoma

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Karen Lichtenfeld, M.D.	Clinical Associate	CL NCI
	Peter H. Wiernik, M.D.	Chief, Clinical Oncology Branch	CL NCI

COOPERATING UNITS (if any)

Department of Surgery, University of Maryland Hospital, Baltimore, Md.

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NCI, NIH Baltimore, Maryland 21201

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1	0.5	0.5

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☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline ke

Although there are too few patients in any arm of this study, the results are similar to those obtained in larger groups, i.e. a 20% response rate to DTIC alone and perhaps a suggestion of a longer response duration in the patient who received immunotherapy. Also there is no evidence to date that adjuvant chemotherapy delays the time to progressive disease. More patients need to be entered into this protocol before completion.

Objectives: to determine the efficacy of DTIC employed as adjuvant therapy in patients with advanced malignant melanoma and in whom all gross disease has been resected and to determine the efficacy of combined chemoimmunotherapy using DTIC and MER in the treatment of patients with advanced non-resectable melanoma.

There have been 25 evaluable patients entered on study. In the adjuvant arm of the protocol, six patients have been randomized to receive no therapy and six patients received DTIC 250 mg/m^2 IV daily times five every three weeks. The median survival from the date of randomization is 173+ days in the no treatment group and 288+ days in the DTIC group. This is not statistically significant. In the second arm of the study, patients with clearly advanced but non-measurable disease are randomized to receive DTIC alone or DTIC plus MER. Thus far only three patients have been entered into this arm and, therefore, cannot be evaluated. A third group of patients with advanced but measurable disease was likewise randomized to receive either DTIC alone or DTIC plus MER. Eight patients received DTIC alone and the median duration of survival was 149+ days. Five patients have received both DTIC plus MER and the median survival is 72+ days. There is no statistical difference between the two groups. Two of eight patients receiving DTIC alone had partial responses lasting 35+ and 21 days, respectively. In the DTIC plus MER gorup, one of five patients had a partial response and this patient lived for 274 days.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 06956-01 COB

PERIOD COVERED

July 1, 1976 through September 30, 1977

TITLE OF PROJECT (80 characters or less)

Measurement of 5-Hydroxyindoleacetic Acid and Beta-2 Microglobulin in Cerebrospinal Fluid of Cancer Patients

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Karen Lichtenfeld, M.D.	Clinical Associate	CL NCI
	Peter H. Wiernik, M.D.	Chief, Clinical Oncology Branch	CL NCI
Others:	Thomas Koch, M.D.		

COOPERATING UNITS (if any)

Department of Clinical Pathology, University of Maryland Hospital, Baltimore, Md.

LAB/BRANCH

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NCI, NIH Baltimore, Maryland 21201

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1	0.5	0.5

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☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline ke

Twenty-one cerebrospinal fluids and corresponding sera have been analyzed for beta-2 microglobulins. This data shows that at least one patient, who had a documented CNS lymphoma, has elevation of the protein in the spinal fluid when compared with the serum. Since the initial patients consist mainly of patients with leukemia, most of whom have had routine lumbar punctures performed to rule out CNS leukemia, it is too early to define the predictive value of beta-2 microglobulin in patients with other malignant diseases. It seems that the protein is not elevated in patients with documented CNS leukemia.

5-Hydroxyindoleacetic acid is currently being evaluated according to the method that we developed. The samples have only been taken through part of the procedure and have been frozen and lyophilized.

This study was undertaken in order to evaluate whether cerebrospinal fluid levels of beta-2 microglobulins or 5-hydroxyindoleacetic acid could serve as markers or predictors for CNS malignancy. It has been shown that 5-hydroxyindoleacetic acid decreases with spinal cord injury. Beta-2 microglobulins have not been studied in the CNS of patients with various disease states.

In evaluating the methods of determination of 5-hydroxyindoleacetic acid, it was found that the various methods were associated with inadequate specificity, sensitivity or simplicity. Therefore, we at first set out to develop a separation method which would permit accurate quantitation of serotonin and 5-hydroxyindoleacetic acid.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 06957-01 COB

PERIOD COVERED
July 1, 1976 through September 30, 1977

TITLE OF PROJECT (80 characters or less)

Nitrous Oxide in the Treatment of Refractory Adult Leukemia

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Karen Lichtenfeld, M.D.	Clinical Associate	CL NCI
	Peter H. Wiernik, M.D.	Chief, Clinical Oncology Branch	CL NCI
Others:	Martin Helrich		

COOPERATING UNITS (if any)
Department of Anesthesiology, University of Maryland Hospital, Baltimore, Md.

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NCI, NIH Baltimore, Maryland 21201

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1	0.5	0.5

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☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline ke

Two patients have been entered into this study thus far. The first patient had a maximum period of inhalation of 2½ hours, which she tolerated extremely well with no unusual or unexpected toxicity. The second patient was given the inhalation for a maximum of six hours which she tolerated equally well. There was no effect on peripheral counts or bone marrow of either patient.

It has been documented that prolonged inhalation of nitrous oxide over a period of four or more days can lead to bone marrow aplasia. Although there is some clinical data suggesting that high peripheral counts and perhaps bone marrow can be suppressed in leukemia patients, there have been no systematic clinical trials. This protocol is a phase I evaluation of nitrous oxide in the treatment of adults who have failed conventional anti-leukemic agents. The objectives of the study are to determine the efficacy of nitrous oxide in suppressing myelopoiesis and to establish guidelines for the administration and dosage of this therapeutic modality.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 06958-01 COB

PERIOD COVERED
July 1, 1976 through September 30, 1977

TITLE OF PROJECT (80 characters or less)
Investigation of Thrombosis in Patients with Brain Tumors

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Karen Lichtenfeld, M.D.	Clinical Assoicate	CL NCI
Others:	Charles A. Schiffer, M.D.	Senior Investigator	CL NCI
	Peter H. Wiernik, M.D.	Chief, Clinical Oncology Branch	CL NCI
	Michael D. Walker, M.D.	Assoicate Director, BCRC	CL NCI

COOPERATING UNITS (if any)
None

LAB/BRANCH
Clinical Oncology Branch

SECTION
Office of the Chief

INSTITUTE AND LOCATION
NCI, NIH Baltimore, Maryland 21201

TOTAL MANYEARS: 1	PROFESSIONAL: 0.5	OTHER: 0.5

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline k

Thus far only six patients have been studied and there are no consistent clotting abnormalities that suggest a "risk" factor for pulmonary embolization. One patient who was ambulatory did have a pulmonary emoblism while being studied, and his clotting parameters prior to this event were within normal limits. Likewise, no patient had venous thrombosis or other clotting abnormalities.

The association of thromboembolic phenomenon with malignant tumors is well documented, but none of the reported series described abnormal clotting in association with primary nervous system neoplasms. We have noted that there seem to be an unusual number of patients with brain tumors who develop pulmonary emboli. Very few of these patients were bedridden and all had normal routine clotting parameters and platelet counts. This project seeks to analyze coagulation parameters in patients with CNS tumors in a prospective fashion. The parameters studied include PT, PTT, fibrinogen, fibrin split products, silicone coagulation, factors V and VIII and platelet aggregation.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 06959-01 COB

PERIOD COVERED
July 1, 1976 through September 30, 1977

TITLE OF PROJECT (80 characters or less)

The Effect of General Anesthetic Agents on Platelet Aggregation

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Karen Lichtenfeld, M.D.	Clinical Associate	CL NCI
	Peter H. Wiernik, M.D.	Chief, Clinical Oncology Branch	CL NCI
Others:	Charles A. Schiffer, M.D.	Senior Investigator	CL NCI
	Martin Helrich		

COOPERATING UNITS (if any)

Department of Anesthesiology, University of Maryland Hospital, Baltimore, Maryland 21201

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Clinical Oncology Branch

SECTION
Office of the Chief

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NCI, NIH Baltimore, Maryland 21201

TOTAL MANYEARS: 1	PROFESSIONAL: 0.5	OTHER: 0.5

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☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline k

Thus far only four patients have been entered on study and there is definite evidence of decreased platelet aggregation in one of these patients. We plan to study 20 patients and then determine whether or not the study should be continued.

PHS-6040
(Rev. 10-76)

Although there is in vitro data suggesting that general anesthetic agents have an inhibitory effect on platelet aggregation, this has never been substantiated in vivo. The current protocol is an evaluation of platelet function in patients undergoing short surgical procedures requiring simple anesthetic agents. Blood is drawn pre-operatively, post-anesthesia induction but prior to surgery, during surgery, and one hour post-operatively. Platelet aggregation is then assessed using a standard aggregometer and standard reagents, including ADP, epinephrine and collagen at varying concentrations.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE
PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF
HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 CM 06960-01 COB

PERIOD COVERED
July 1, 1976 through September 30, 1977

TITLE OF PROJECT (80 characters or less)
New Drug Therapy of Previously Treated Adult Leukemia

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	David A. Van Echo, M.D.	Clinical Associate	CL NCI
	Peter H. Wiernik, M.D.	Chief, Clinical Oncology Branch	CL NCI

COOPERATING UNITS (if any)
None

LAB/BRANCH
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NCI, NIH Baltimore, Maryland 21201

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1	1	0

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☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline k

The result of this project indicates that:

1. the combination of vinblastine, azacytidine and VP 16-213 is not more effective or toxic than azacytidine alone in the treatment of ANLL, and that

2. neocarzinostatin has little antileukemic activity at the dosages employed in this study but can cause prolonged bone marrow aplasia in some patients.

PHS-6040
(Rev. 10-76)

Project Description:

PROJECT A

To determine whether the combination of 5-azacytidine, vinblastine, VP 16-213 (VAV) or 5-azacytidine, VP 16-213 (AV) could improve on the results achieved with 5-azacytidine alone.

Twenty-seven patients with acute nonlymphocytic leukemia, who had received extensive prior therapy, were included in this study. The first 15 patients received the VAV combination and only two patients responded. One patient achieved a complete remission on two occasions for 56 and 42 days, respectively. The second patient achieved a short complete remission for 46 days and a partial remission for 10 days. The next 12 patients received the AV combination with higher doses of the two drugs than in the VAV combination. In addition, two patients treated previously with VAV were given AV treated group. Toxicities in both groups were comparable and although considerable, these toxicities were not considered any more extensive than regimens employing 5-azacytidine alone in full dosage. The main toxicity was hematologic, i.e., leukopenia and bone marrow suppression. Non-hematologic toxicities included: nausea and vomiting, weakness, lethargy, pharyngitis, esophagitis and fever. Because of the poor responses, these combinations are not felt to be suitable for further study.

Study closed to patient entry and other combination studies with 5-azacytidine are about to begin.

PROJECT B

To determine the toxicity and possible therapeutic effectiveness of neocarzinostatin in patients with refractory acute leukemia.

Fifteen adult patients with acute nonlymphocytic leukemia and one child with acute lymphocytic leukemia, who had received extensive therapy and were refractory to conventional agents, were included in this study. The drug was given as an one-hour infusion in a foil-covered bottle on five consecutive days. Courses of therapy were repeated at 2 to 3 week intervals depending on bone marrow recovery. The initial dosage was 1,000 IU/m^2/dx5. This was escalated in an orderly manner to 3,500 IU/m^2/dx15. Side effects included: nausea and vomiting, 10 patients; fever and chills, 4 patients (prevented by premedication with tylenol and benadryl); hypotension (secondary to high fever and dehydration), 1 patient. There was no evidence of hepatic, renal or CNS toxicity. Bone marrow suppression occurred sporadically at dosages at or above 2,000 IU/m^2/dx5. Hypoplasia occured at 2,000 IU/m^2/dx5, 2,500 IU/m^2/dx10 and 3,000 IU/m^2/dx10. Severe and prolonged aplasia occurred at 3,000 IU/m^2/dx10 and 3,500 IU/m^2/dx10. There were other patients at these dosages or higher who had no bone marrow effects whatsoever. No antileukemic responses were seen in the adult patients, but the child achieved a short one month partial remission. Because of the poor responses and unpredictable toxicity, it is felt this drug has limited potential for further study. Study closed to patient entry.

Publications:

1. Van Echo, D.A., Lichtenfeld, K.M. and Wiernik, P.H.: Vinblastine, 5-azacytidine and VP 16-213 therapy in previously treated patients with acute nonlymphocytic leukemia. Cancer Treatment Reports, 1977 (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 06961-01 COB

PERIOD COVERED
July 1, 1976 through September 30, 1977

TITLE OF PROJECT (80 characters or less)

Colony Stimulating Factor and Colony Forming Capacity from Patients with Neoplasms

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	David A. Van Echo, M.D.	Clinical Associate	CL NCI
	Peter H. Wienrik, M.D.	Chief, Clinical Oncology Branch	CL NCI

COOPERATING UNITS (if any)
None

LAB/BRANCH
Clinical Oncology Branch

SECTION
Office of the Chief

INSTITUTE AND LOCATION
NCI, NIH Baltimore, Maryland 21201

TOTAL MANYEARS: 1	PROFESSIONAL: 1	OTHER: 0

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☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline k

It has been determined in this study that colony forming units are decreased or absent in patients with acute nonlymphocytic leukemia, no matter what disease stage.

PHS-6040
(Rev. 10-76)

To determine the amount of colony-stimulating factor (CSF) and colony-forming units (CFU) in various states of acute leukemia and morphologic studies of in vitro grown leukemic cells.

The growth technique involves incubation for 7-14 days of bone marrow cells obtained from mice or humans in 35 mm petri dishes containing a semi-solid culture medium at $37^{\circ}C$ in a CO_2 humidified incubator. The dishes are removed and examined under a dissecting microscope for cell morphology and cluster or colony formation. The culture media is made up of a mixture of methylcellulose, fetal calf serum, McCoy's media, essential amino acids and antibiotics (penicillin and streptomycin).

Although in our labs the growth technique was quite successful for growing cells from normal mice or human bone marrow, cells from patients with lymphoma, chronic myelogenous leukemia, ovarian carcinoma or cultured lines of lymphoblasts, the technique proved inadequate to obtain growth of colonies of cells from 16 patients with acute nonlymphocytic leukemia. Various maneuvers including higher concentrations of stimulant (standard CSF source), Feeder layers, additives that have been reported to be successful in the literature such as vitamin C and pythohemagglutinin were all unsuccessful. It made no difference whether the cells were obtained from a patient in remission, relapse or prior to first induction.

It is hoped that further developments in culture techniques will allow more detailed studies of hematopoietic function to be undertaken.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE
PROJECT NUMBER (Do **NOT** use this space)

U.S. DEPARTMENT OF
HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 CM-06217-08 COB

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Prevention of Infections in Patients with Cancer

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	S.C. Schimpff	Head, Section of Infection Research	CL NCI
OTHER:	J. Aisner	Senior Investigator	CL NCI
	M. Brouillet	Infection Control Nurse	CL NCI
	D.M. Hahn	Clinical Associate	CL NCI
	P.H. Wiernik	Chief, Clinical Oncology Branch	CL NCI

COOPERATING UNITS (if any)
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C.L. Fortner Clinical Research Pharmacy
R.B. Hornick; M.J. Snyder Division of Infectious Diseases, University of Maryland

LAB/BRANCH
Clinical Oncology Branch

SECTION
Section of Infection Research

INSTITUTE AND LOCATION
NCI, NIH Baltimore, Maryland 21201

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
9	6.5	2.5

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☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline ke

Infection remains as a major cause of both morbidity and mortality for patients with various types of cancers. The risk of infection increases as new techniques are introduced into medical practice and as greater numbers of patients are treated with more intensive cytotoxic and immunosuppressive therapy. It is therefore essential in the modern practice of oncology that definitive approaches be designed and evaluated to reduce the opportunities for infection. At the Baltimore Cancer Research Center an intensive infection surveillance program assesses the ongoing infectious disease status of the patients and the Center as a whole. From this data base have grown appropriate basic infection control policies and specific research approaches including evaluation of laminar air flow rooms, simpler means of air filtration and reverse isolation, oral nonabsorbable antibiotics and techniques to prevent axillary and oral infections. These programs are enforced by the infection control nurse. A major program has been the reduction of an environmental source of fungi which had contributed to frequent Aspergillus infections. The net result of these varied approaches has been a very substantial reduction in infections among patients at this institution.

PHS-6040
(Rev. 10-76)

Infection remains as a major cause of both morbidity and mortality for patients with various types of cancers. The risk of infection increases as new techniques are introduced into medical practice and as greater numbers of patients are treated with more intensive cytotoxic and immunosuppressive therapy. It is therefore essential in the modern practice of oncology that definitive approaches be designed and evaluated to reduce the opportunities for infection.

A) Surveillance - The cornerstone of an infection control program is an accurate understanding of the infections presently occurring including the sites and etiologic agents, apparent predisposing factors, routes of acquisition of hospital organisms, and changing patterns of morbidity and mortality. At the Baltimore Cancer Research Center, this is accomplished by the daily rounds of an infection control nurse, twice weekly infectious disease rounds, daily evaluation of all infected or potentially infected patients, and an extensive program of both patient and environmental microbial culturing. The data derived is summarized regularly and utilized to adjust, on a continuing basis, the programs in infection control.

B) Basic Infection Control Procedures - The Infection Control Policy Manual has been continually updated with enforcement persued by the infection control nurse. The following procedures are standard: 1) urinary, intravenous and hyperalimentation catheters are avoided except under extenuating circumstances, 2) all intravenous solutions are prepared in laminar air flow hoods, all bottles and tubing are replaced daily, and butterfly needles are changed every other day, 3) personnel hygiene is emphasized including handwashing and the use of special germicidal hand lotions, 4) all inpatients are seen regularly by the infection control nurse for discussion of preventive techniques, 5) results of patient and environmental surveillance cultures are utilized to direct specific control techniques. These techniques have substantially reduced nosocomial infection.

C) Oral Nonabsorbable Antibiotics - Controlled trials at the BCRC and elsewhere have indicated the usefulness of oral nonabsorbable antibiotics in reducing the incidence of severe infections arising along the alimentary tract and also in the lungs. Although not due solely to the oral antibiotics, the incidence of bacteremia has fallen from 13.3 per 1000 days to 3.2 per 1000 days over the past 5 years. However, the poor taste and frequent nausea and diarrhea leads to difficulties with compliance and hence efficacy. A series of comparative trials was therefore initiated comparing taste, tolerance, compliance, microbial suppression and infection prevention. A double-blind "tasting" by 31 individuals ranked nystatin(N), gentamicin(G), tobramycin(T), amphotericin B(A) and vancomycin(V) liquid in that order from "good-neutral" to "totally unacceptable". To date, 32 consecutive patients with acute nonlymphocytic leukemia in relapse have been randomized to receive, single-blind, GVN or GN. Surveillance cultures are obtained twice weekly. A nurse and pharmacist educate patients while recording tolerance and compliance. Tolerance is much better to the combination without vancomycin although compliance is similar with excellent suppression of rectal microbes when taken regularly. Also to date, 30 consecutive newly admitted acute nonlymphocytic leukemia patients have been randomized, double-blind, to GVN or TVN. Both are about equally tolerable with similar compliance, microbial suppression and infection prophylaxis. Indeed, provided the agents are taken regularly, the microbial suppression is excellent and infection incidence is low. This study indicates that tobramycin can be substituted for gentamicin but data is still insufficient to determine if the vancomycin can be discontinued without loss of efficacy.

Another approach to the problem of compliance is to improve tolerance by adjusting taste. Through a contract with Arthur D. Little, Company, a panel of taste experts is attempting to produce a "neutral" taste to vancomycin, gentamicin, tobramycin and nystatin. These taste-adjusted drugs will be compared with the current formulation to determine patient tolerance and compliance. Through these two approaches (substitution or discontinuation of drugs and taste modification) it may be possible to improve compliance and hence reduce infection. The study is therefore continuing.

D) Evaluation of Reverse Isolation in Standard Hospital Rooms with or without Added Air Filtration - Forty-two uninfected, previously untreated patients with acute nonlymphocytic leukemia were randomly allocated to one of three groups to evaluate whether a basic program of infection prevention plus complete reverse isolation with added air filtration (Group I) or the basic program plus reverse isolation without added air filtration (Group II) would be more efficacious for infection prevention than the basic program alone which included a cooked food diet, patient and staff hygiene education and oral nonabsorbable antibiotics (Group III). The patient populations in the three groups were comparable. Environmental culture results were similar among the three groups and indicated minimal contamination. There were no substantial differences in new organism acquisition, infection incidence, infection site or pathogen, infectious deaths, hematologic remission or survival among these three groups in the setting of a modern hospital with good microbiologic environmental control. An additional 16 patients (Group IV), admitted with infection, were treated equivalently to control Group III. Their rate of organism acquisition and infection incidence was equivalent to those of the other patients but infectious deaths were more common resulting in fewer remissions and a lessened survival. This study suggests, in the setting of a modern hospital with prefiltered, nonrecirculating air, good housekeeping routines and careful attention to basic concepts of infection control including cooked food diet and oral nonabsorbable antibiotics, that the addition of reverse isolation with or without added air filtration in standard rooms will not measurably add to infection prevention. This does not preclude, however, the potential efficacy of total reverse isolation as can be accomplished in the setting of a laminar air flow room.

E) Aspergillus Pneumonia - After the BCRC moved to a new hospital location in 1974, an increase in respiratory tract Aspergillus flavus infection was documented unrelated to changes in disease therapy or supportive care. An extensive search revealed that the steel-beam fireproofing material was contaminated with various fungal organisms including Aspergillus flavus, fumigatus and niger. A fungicidal agent, copper-8-quinolinolate, was evaluated by spraying all fireproofing and false ceiling tiles in a test room. Fireproofing material and false ceiling cultures revealed extensive fungi before but not after application and air sample colony counts after application were reduced compared to the control period. As a result, all fireproofing material and involved false ceilings was treated with this compound in May, 1976. Cultures similar to those of the test room showed marked reduction in fungal contamination. No Aspergillus infections have occurred among BCRC patients during the 12 months following copper-8-quinolinolate application.

F) Prevention of Axillary Lesions - Axillary lesions are common in granulocytopenic patients with acute leukemia. Personal hygiene, avoidance of occlusive antiperspirants and shaving only with an electric razor will partially reduce the frequency of these infections which are most commonly caused by gram-negative bacilli as well as by Staphylococcus aureus. In 1975, a program was instituted to have each leukemia patient swab their axilla four times per day with a povidone-iodine swabstick. Since that time only two patients have developed these lesions; both patients had discontinued use of the iodine swabstick preparation. This simple, inexpensive technique has markedly reduced a previously common cause of infectious morbidity.

G) Prevention of Oral Lesions - Periodontal infection is common in granulocytopenic patients. In an attempt to reduce this complication, BCRC patients are evaluated by an oral surgeon for reparative needs and have an oral-dental prophylaxis and dental hygiene education completed by a dental hygienist with follow-up by the infection control nurse. Although only recently instituted, this program would appear to be reducing the oral complications of chemotherapy.

Publications:

Schimpff, S.C.: Laminar air flow room reverse isolation and microbial suppression to prevent infection in patients with cancer. Cancer Chemother. Rep. 59:1055-1060, 1975.

Aisner, J., Schimpff, S.C., Bennett, J.E., Young, V.M. and Wiernik, P.H.: Aspergillus infections in cancer patients. Association with fireproofing materials in a new hospital. JAMA 235:411-412, 1976.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01-CM-06264-08 COB

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Evaluation of the Types and Causes of Infectious Diseases in Cancer Patients

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	S.C. Schimpff	Head, Section of Infection Research	CL NCI
OTHER:	J. Aisner	Senior Investigator	CL NCI
	M. Brouillet	Infection Control Nurse	CL NCI
	D.M. Hahn	Clinical Associate	CL NCI
	P.H. Wiernik	Chief, Clinical Oncology Branch	CL NCI
	P. Espana	Guest Worker	CL NCI

COOPERATING UNITS (if any)

V.M. Young Research Microbiology Section

LAB/BRANCH
Clinical Oncology Branch

SECTION
Section of Infection Research

INSTITUTE AND LOCATION
NCI, NIH Baltimore, Maryland 21201

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.5	3.0	.50

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline ke

Infection is the leading cause of morbidity and mortality in patients with acute leukemia and a major cause in other types of advanced malignancies. The aims of this project are to catalogue and identify the infections, identify the predisposing factors, and evaluate new techniques for more rapid and/or accurate diagnosis of infection. This program has been in progress for over 7 years and has allowed meaningful evaluation of the types of infection occurring in different types of tumor patients. Further, specific problems such as environmental contamination with fungi such as *Aspergillus* and recognition of patterns of pathogen acquisition such as *Pseudomonas aeruginosa* and *Staphylococcus aureus* have been identified such that appropriate intensive preventive measures could be devised and enforced with consequent marked reduction in infection morbidity.

Infection is the leading cause of morbidity and mortality in patients with acute leukemia and a major cause in other types of advanced malignancies. The aims of this project are to 1) catalogue and identify the infections manifest both clinically and postmortem, 2) identify the predisposing factors which have contributed to the specific type of infection, 3) evaluate new techniques for more rapid and/or accurate diagnosis of infection.

Beginning July 1, 1970, each patient with suspected infection has been seen by one of the investigators who examines the patient and reviews the chart, both initially and throughout the course of the infection. Detailed records are kept of history, examination, laboratory data, etc. Similar details are obtained on infections detected at the time of postmortem examination. Formal infectious disease rounds are held twice weekly to review each recognized infection, discuss management and evaluate how each infection might have been prevented.

A) Infection Develops Depending on Predisposing Factors - Review of the infections and their settings over the past seven years reveals certain distinct patterns.

1) Granulocytopenic patients (e.g., acute leukemia) develop pneumonia, perianal lesions, pharyngitis and skin lesions commonly and these infections are usually caused by gram-negative bacilli and _S. aureus_. Fungal infections (_Candida_, _Torulopsis_, and _Aspergillus_) occur in the patient with prior long-term use of broad spectrum antibiotics.

2) Patients with deficits in cellular immunity (e.g., lymphoma) develop infections caused by the bacteria _Listeria_, _Salmonella_, _Mycobacterium_ and _Nocardia_, the viruses Varicella-zoster and cytomegalovirus, the yeast _Cryptococcus_ and the protozoa _Toxoplasma_ and _Pneumocystis_.

3) Patients with CNS dysfunction (e.g., brain tumor) develop pneumonia due to aspirated oral flora and urinary tract infection due to impaired micturition.

4) Patients with lesions obstructing natural passages develop post-obstructive infection (e.g., bronchogenic carcinoma and pneumonia).

Recognition of these patterns allows for more appropriate use of diagnostic techniques and aids in the selection of appropriate therapy before culture results are known.

B) _Aspergillus_ Infection - A sudden increase in the frequency of _Aspergillus flavus_ infection provided a setting to evaluate the epidemiology of this infection. This evaluation indicated that _Aspergillus_ spores reach the patient via airborne routes and can colonize the upper respiratory tract provided the normal nasal bacterial flora has been suppressed by antibacterial antibiotics. Colonization can progress to infection in the patient with persistent granulocytopenia. Infection can probably be prevented by interrupting any point in the process, i.e., airborne contamination (see #6950), suppression of normal nasal flora, elimination of colonization or return of peripheral granulocytes.

C) Hospital Acquisition of Organisms - Acquired organisms represent the source of about 50% of the infecting pathogens in patients with acute leukemia. At the BCRC, surveillance cultures of nose, axilla, gingiva and rectum are taken at admission and then twice weekly. Ninety-one consecutive patients were analyzed to determine how many bacteremias were caused by the patient's own endogenous flora present at admission as compared to the bacteremias caused by hospital-acquired organisms. In these patients, studied over four years, 32 bacteremias were due to endogenous flora whereas 41 were hospital-acquired pathogens. The implication of this study is that efforts to reduce hospital acquisition of potential pathogens are essential to the management of adult patients with acute leukemia.

Publications:

Aisner, J., Schimpff, S.C., Bennett, J.E., Young, V.M. and Wiernik, P.H.: Aspergillus infections in cancer patients. Association with fireproofing materials in a new hospital. JAMA 235:411-412, 1976.

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PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Therapy of Suspected Bacterial Infection in Granulocytopenic Patients with Cancer

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	S.C. Schimpff	Head, Section of Infection Research	CL NCI
OTHER:	D.M. Hahn	Clinical Associate	CL NCI
	J. Aisner	Senior Investigator	CL NCI
	C.A. Schiffer	Senior Investigator	CL NCI
	P.H. Wiernik	Chief, Clinical Oncology Branch	CL NCI

COOPERATING UNITS (if any)
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V.M. Young; N. Friedman Research Microbiology Section
H. Standiford Division of Infectious Diseases, University of Maryland
European Organization for Research on Treatment of Cancer, Antimicrobial Therapy Project Group

LAB/BRANCH
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SECTION
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INSTITUTE AND LOCATION
NCI, NIH Baltimore, Maryland 21201

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2	1.5	.50

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline k

A series of consecutive antibiotic trials for suspected sepsis in granulocytopenic cancer patients began in 1969 and continues to the present. These evaluations have included joint ventures with the International Antimicrobial Therapy Project Group of the EORTC in a study which indicated that carbenicillin plus gentamicin had the best efficacy to toxicity ratio of the three possible two-drug combinations of carbenicillin, gentamicin, and cephalothin. The nephrotoxic potential of cephalothin and gentamicin has been proven yet methods to limit such toxicity have also been demonstrated with the combination of cephalothin and amikacin. Ticarcillin has been proven equally efficacious to carbenicillin at 1/2 to 2/3 of the carbenicillin dosage. Currently, gentamicin, amikacin and netilmicin, each in combination with ticarcillin, are being compared for efficacy and toxicity. When utilized with early granulocyte transfusions for poor risk patients, these agents have been highly effective with no nephrotoxicity in 92 consecutive patient-trials.

PHS-6040
(Rev. 10-76)

Despite improvements in preventive techniques, infections remain as one of the most common serious complications of cancer therapy. The BCRC Section of Infection Research has continuing programs designed to improve the therapeutic approach to these infections.

A) Empiric Antibiotic Therapy for the Febrile Granulocytopenic Patient- The setting of new fever during granulocytopenia represents infection in ∿70% of cases with bacteremia occurring in 20% thus necessitating the prompt empiric use of broad spectrum, bactericidal antibiotics.

1) The BCRC has helped to form and participate in a multiinstitutional international antimicrobial therapy trial under the auspices of the European Organization for Research on Treatment of Cancer (EORTC). Patients were randomly allocated to receive one of three antibiotic combinations as part of a prospective controlled trial. 625 patient-trials (253 from the BCRC) were conducted with approximately 50% of gram-negative bacillary bacteremias and 90% of all other infections responding to the initial antibiotic therapy alone. Carbenicillin plus gentamicin was found to be the combination with the best efficacy to toxicity ratio, showing excellent efficacy for Pseudomonas aeruginosa, Escherichia coli and Staphylococcus aureus infections. Cephalothin plus gentamicin was found to be inadequate for P. aeruginosa bacteremia and to have an unacceptably high order of associated nephrotoxicity (27%) in patients over age 40 years.

2) Ticarcillin, an analog of carbenicillin, has been used in over 200 patient-trials at the BCRC. It has been found to be equally efficacious to carbenicillin but, because it can be utilized at one half the dosage of carbenicillin, has less dose related side effects. As a result, ticarcillin has supplanted carbenicillin and ticarcillin in combination with gentamicin, has become the treatment of choice for P. aeruginosa infection and the treatment of choice as initial empiric therapy for suspected sepsis in febrile granulocytopenic patients.

3) Amikacin, a recently released aminoglycoside, has activity similar to gentamicin but is not a good substrate for 5 of the 6 bacterial enzymes that can inactivate gentamicin. Amikacin was evaluated in combination with cephalothin in 93 febrile granulocytopenic patients. Granulocyte transfusions were begun promptly for patients with poor prognostic factors (aplastic bone marrow in association with gram-negative sepsis or pneumonia). Thirty-eight of 47 infections responded completely as did 11 of 17 bacteremias. This represents the best response rate reported to date and relates to a combination of prompt use of active antibiotics in association with prompt use of granulocyte transfusions in poor risk patients before infection progression (see report #6275). Renal toxicity was 7%; this low incidence was probably related to improved monitoring with rapid dosage adjustment when indicated.

4) Netilmicin is an investigational aminoglycoside similar to gentamicin in activity but having substantially less renal toxic effects in animal studies. A three arm, prospective controlled trial comparing gentamicin to amikacin to netilmicin, each in combination with ticarcillin, was instituted to compare efficacy and toxicity in the febrile granulocytopenic cancer patient. Ninety-two patient-trials have been conducted to date. Granulocyte transfusions were utilized as described above for poor risk patients before infection progression. The three regimens have thus far proven equivalent with excellent response rates for all bacterial infections (92% improvement) and for bacteremias (84%). No patient has developed nephrotoxicity with any of the three combinations. This study is continuing.

5) Serum bactericidal assays have been suggested as a means of predicting success or failure of antibiotic therapy in severe infection. Patients at the BCRC who were part of the EORTC trial had bactericidal assays to 11 strains each of *P. aeruginosa*, *K. pneumoniae*, *E. coli*, and *S. aureus* performed on serum samples before and after antibiotic administration. The in vitro results paralleled the ultimate in vivo efficacy of the three antibiotic combinations, i.e., ticarcillin plus gentamicin was, overall, the best combination and cephalothin plus gentamicin was inadequate for *P. aeruginosa*. This approach will therefore be continued with the current ticarcillin plus either gentamicin, amikacin or netilmicin evaluation.

B) Evaluation of Low Dose Radiation as Adjunctive Therapy to the Management of Localized Skin and Perianal Lesions in Patients with Acute Leukemia.

Primary skin infections are an important source from which disseminated sepsis may arise in granulocytopenic patients. Uncontrolled trials have suggested that low doses of megavoltage radiation therapy may assist in controlling such lesions. A prospective, double-blind randomized trial was therefore established to evaluate the possible efficacy of radiation therapy in this setting. Thirty-five perianal, axillary or other skin lesions in granulocytopenic patients with acute leukemia were treated with (17) or without (18) 400 rad megavoltage radiation therapy on day 1 and day 8 after lesion identification. All patients were treated with the same or similar broad spectrum bactericidal antibiotic combinations as discussed in A above.

The overall complete and partial response rates were good but no differences in response rate or median time to complete response were noted between the two groups.

It is concluded that low dose megavoltage radiation, as used in this study, did not contribute toward improved responses. The study has been concluded.

Gaya, H., Klastersky, J., Schimpff, S.C., Fiere, D., Widmaier, S. and Nagel, G.: Prospective randomly controlled trial of three antibiotic combinations for empirical therapy of suspected sepsis in neutropenic cancer patients. Europ. J. Cancer 11(Suppl.):S5-S8, 1975.

Gaya, H., Klastersky, J., Schimpff, S.C. and Tattersall, M.H.N.: Protocol for an international prospective trial of initial therapy regimens in neutropenic patients with malignant disease. Europ. J. Cancer 11(Suppl.):S1-S4, 1975.

EORTC International Antimicrobial Therapy Project Group: Infection in febrile granulocytopenic patients with cancer. J. Inf. Dis. (in press).

Schimpff, S.C., Landesman, S., Hahn, D.M., Standiford, H.C., Fortner, C.L., Young, V.M. and Wiernik, P.H.: Ticarcillin in combination with cephalothin or gentamicin as empiric antibiotic therapy in granulocytopenic cancer patients. Antimicrob. Ag. Chemother. 10:837-844, 1976.

Lopez, C.E., Standiford, H.C., Tatem, B.A., Calia, F.M., Schimpff, S.C., Snyder, M.J. and Hornick, R.B.: Pirbenicillin: comparison with carbenicillin and BL-P1654, alone and with gentamicin, against Pseudomonas aeruginosa. Antimicrob. Ag. Chemother. 11:441-448, 1977.

Schimpff, S.C.: Therapy of infection in patients with granulocytopenia. Med. Clin. No. Amer. (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01-CM-06922-05 COB

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Epidemiologic Investigations of Patients with Leukemia and Lymphoma

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	S.C. Schimpff	Head, Section of Infection Research	CL NCI
OTHER:	D.A. Brager	Research Assistant	CL NCI
	P.H. Wiernik	Chief, Clinical Oncology Branch	CL NCI

COOPERATING UNITS (if any)

G. Comstock Johns Hopkins University School of Public Health and Hygiene

LAB/BRANCH
Clinical Oncology Branch

SECTION
Section of Infection Research

INSTITUTE AND LOCATION
NCI, NIH Baltimore, Maryland 21201

TOTAL MANYEARS: 0.5	PROFESSIONAL: .25	OTHER: .25

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline key

Recent published observations have suggested that some patients with Hodgkin's disease may have been close personal contacts with others who subsequently developed the disease. The BCRC investigation was instituted to assess the possible relevance of interpersonal contact in the epidemiology of both leukemia and lymphoma. Following initial evaluations which suggested a high frequency of personal interactions among patients prior to diagnosis, two separate areas were then chosen for case-control evaluation of social linkages. In one of the two areas, the cases had significantly more close personal associations than expected thus supporting, in part, the previous observations of social linkages between patients. These evaluations are being extended.

Project Description:

Prior Social Interaction Among Patients with Leukemia and Lymphoma - Recent published observations have suggested that some patients with Hodgkin's disease may have been close personal contacts with others who subsequently developed the disease. The BCRC investigation was instituted to assess the possible relevance of interpersonal contact in the epidemiology of both leukemia and lymphoma. Each case report of leukemia and lymphoma for a 10-year period from three areas of West Virginia was obtained and the patient or a surviving friend or relative was interviewed. In each area, over 60% of all cases could be interlinked on the basis of prior close personal interactions. Similar findings were obtained from two areas in Maryland and one in Pennsylvania.

Two separate areas were then chosen for case-control evaluation of social linkages. From each area, every patient with leukemia or lymphoma was ascertained through the tumor registries. Controls were randomly selected, matched for age, sex, and residence as of date of diagnosis. Social linkages between cases, controls and cases plus controls were ascertained. In one of the two areas, the cases had significantly more close personal associations than expected thus supporting, in part, the previous observations of social linkages between patients. This case-control approach will be extended to other areas.

Current study includes personal interviews with one hundred consecutive patients with Hodgkin's disease and a smaller number of patients with acute leukemia. Interviews and subsequent medical record checks are designed to determine the frequency of related and unrelated personal contacts with the same or similar disease.

Publications:

Schimpff, S.C., Brager, D.M., Schimpff, C.R., Comstock, G.W., and Wiernik, P.H.: Leukemia and lymphoma patients linked by prior social contact. Evaluation utilizing a case-control approach. Ann. Intern. Med. 84:547-550, 1976.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM-06950-01 COB

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Therapy of Fungal Infections in Patients with Cancer

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. Aisner	Senior Investigator	CL NCI
OTHER:	S.C. Schimpff	Head, Section of Infection Research	CL NCI

COOPERATING UNITS (if any)

LAB/BRANCH
Clinical Oncology Branch

SECTION
Section of Infection Research

INSTITUTE AND LOCATION
NCI, NIH Baltimore, Maryland 21201

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	0

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline k

Fungal infections in cancer patients are difficult to diagnose and such diagnoses are frequently only made at necropsy. Aspergillus infections were common at the BCRC (see #6264 regarding prevention) and were usually fatal, but as diagnostic procedures improved, earlier therapy became possible. Therefore, 17 consecutive patients with documented aspergillosis were reviewed to determine the impact of earlier diagnosis and prompt treatment with amphotericin B. Sixteen of the 17 had hematologic malignancies, and all had marked granulocytopenia. Six patients were diagnosed and treated within 96 hours of the appearance of infiltrates; three had complete resolution and three had a partial response to therapy despite continued granulocytopenia. Thus, aggressive diagnostic techniques are warranted. We are now evaluating the addition of rifampin to amphotericin B, known to be synergistic against some fungi in animal models, for early therapy. Nineteen patients have been randomly allocated to date to amphotericin B ± rifampin, but these are too few patients from which to draw meaningful conclusions other than that early application of therapy continues to be the best approach to infection resolution.

PHS-6040
(Rev. 10-76)

Infections with Candida sp, Torulopsis glabrata, Aspergillus sp and Phycomycete (Mucor) sp are increasing as more patients survive initial bacterial infections. Unfortunately, amphotericin B is the only antibiotic of defined efficacy yet the present response rate is low. Recently rifampin has been shown in vitro to be synergistic with amphotericin B against these yeast and fungal organisms. The BCRC has therefore begun a prospective randomized trial of amphotericin B versus amphotericin B plus rifampin as initial therapy for proven or strongly suspected fungal infection. The study will continue.

A) Early Therapy of Invasive Aspergillus Infection - Aspergillus infections in cancer patients are difficult to diagnose and such diagnoses are frequently made at necropsy. Earlier therapy has been proposed to provide better response. Therefore, 17 consecutive patients with documented aspergillosis were reviewed to determine the impact of earlier diagnosis and prompt treatment with amphotericin B. Sixteen of the 17 had hematologic malignancies, and all had marked granulocytopenia. Six patients were diagnosed and treated within 96 hours of the appearance of infiltrates. Three of these 6 had complete resolution of all signs and symptoms of Aspergillus infection. The other three had a partial response to therapy despite continued granulocytopenia. All 11 patients in whom antifungal therapy was either delayed (6) or not given (5) for at least 2 weeks after the infiltrate was present, died with progressive aspergillosis. Aggressive diagnostic techniques to establish the diagnosis of aspergillosis are warranted so that early institution of antifungal therapy can be instituted, which may then be successful in resolving these potentially fatal infections. This study has been completed and has lead to the therapeutic evaluation described below.

B) Evaluation of Rifampin in Combination with Amphotericin B - Infections with Candida sp, Torulopsis glabrata, Aspergillus sp and Phycomycete (Mucor) sp are increasing as more patients survive initial bacterial infections. Unfortunately, amphotericin B is the only antibiotic of defined efficacy yet the present response rate is low. Recently rifampin has been shown in vitro to be synergistic with amphotericin B against these yeast and fungal organisms. The BCRC has therefore begun a prospective randomized trial of amphotericin B versus amphotericin B plus rifampin as initial therapy for proven or strongly suspected fungal infection. Nineteen patients have been randomized to date (nine to amphotericin B alone and ten to amphotericin B plus rifampin); this represents too few patients to show any difference, should a difference exist, between the two regimens. The study will continue.

Publications:

Aisner, J., Schimpff, S.C., and Wiernik, P.H.: Treatment of invasive aspergillosis: relation of early diagnosis and treatment to response. Ann. Intern. Med. 86:539-543, 1977.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01-CM-06951-01 COB

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Infection Research in Oncology

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	S.C. Schimpff	Head, Section of Infection Research	CL NCI
OTHER:	D.M. Hahn	Clinical Associate	CL NCI
	J. Aisner	Senior Investigator	CL NCI
	M. Brouillet	Infection Control Nurse	CL NCI
	P.H. Wiernik	Chief, Clinical Oncology Branch	CL NCI
	V.M. Young	Head, Research Microbiology Section	CL NCI
	C.A. Schiffer	Senior Investigator	CL NCI

COOPERATING UNITS (if any)
H. Standiford; R. Hornick Division of Infectious Diseases, University of Maryland Hospital

LAB/BRANCH
Clinical Oncology Branch

SECTION
Section of Infection Research

INSTITUTE AND LOCATION
NCI, NIH Baltimore, Maryland 21201

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.5	1.0	0.5

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline ke

Infection is the leading cause of morbidity and mortality in patients with acute leukemia and a major cause in other types of advanced malignancies. The aim of this program is an integration of research, education, and patient care with regard to the infections arising in patients with cancer. The research areas include infection epidemiology, diagnosis, prevention and treatment. Education includes the teaching of BCRC clinical associates the fundamental problems and techniques for infection management, teaching the nursing staff basic prevention and treatment modalities, and teaching the University of Maryland Infectious Disease fellows and students concepts of infection control in the tumor patient. Patient care includes patient education in infection prevention in addition to infection diagnosis, prevention and management.

PHS-6040
(Rev. 10-76)

The frequency and severity of infection in patients with advanced cancer necessitates an ongoing program of 1) infection research, 2) education and 3) patient care. The aims of this program are to integrate these three areas into the ongoing clinical and laboratory programs at the BCRC.

Research areas include infection epidemiology, diagnosis, prevention and therapy (see #6264, #6266, #6950, and #6217).

Education includes the training of BCRC clinical associates, University of Maryland Infectious Disease fellows, and Department of Medicine rotating residents in the management approaches necessary for optimum care of the infected or potentially infected cancer patient. This is done through the daily work rounds plus informal discussions. Nursing education, done largely by the Infection Control Nurse, is designed around twice monthly teaching conferences plus informal patient management discussions.

An integrated team approach has been developed, based around twice weekly formal infection rounds, and includes active participation by physicians from the Infection Research section and the Cell Support program, the infection control nurse, microbiologist and pharmacist. This approach ensures thorough discussion of the various management approaches for each patient while reviewing the requirements of protocol studies.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 06027-08 COB

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Microbiological Services for Clinical and Research Studies of Cancer Patients

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Viola Mae Young, Ph.D.	Head, Microbiology Section	CL NCI
Others:	Marcia R. Moody	Research Microbiologist	CL NCI
	Arthur S. Levine, M.D.	Chief, Ped. Oncology	COP DCT NCI
	Phillip A. Pizzo, M.D.	Senior Investigator	COP DCT NCI
	Norman Friedman	Microbiologist	CL NCI
	Michael T. Hargadon	Microbiologist	Hazleton Labs.
	Maureen J. Morris	Lab. Scientist	CL NCI
	George Fukui, Ph.D.	Director, Microbiology	Hazleton Labs.
	John Parker, Ph.D.	Principal Investigator	Microbiological Associates

COOPERATING UNITS (if any) Pediatric Oncology Branch, COP, DCT, NCI, Bethesda, Md.
Hazleton Laboratories, Vienna, Va.
Diagnostic Laboratory, Microbiological Associates, Walkersville, Md.
Section of Infection Research, COB, BCRC

LAB/BRANCH
Clinical Oncology

SECTION
Microbiology

INSTITUTE AND LOCATION
NCI, NIH Baltimore, Md. 21201

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1-3/4	1-1/2	1/4 Additional man-years are contracted.

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline ke

This project continues to provide high quality clinical diagnostic microbiology to discover bacteria, mycoplasma, and fungi which cause infection in cancer patients. In addition, the presence of viruses are investigated and rises in viral serum antibodies are determined. Initial surveillance cultures determine the patients' flora upon admission to the hospital, and acquisition of potentially pathogenic organisms during the hospital stay is determined by surveillance cultures from high risk patients that are repeated on a routine basis. Cultures of the environment enable the evaluation of cleaning procedures, the detection of "shedders" of pathogenic organisms, and also provide data on the efficacy of protective environments. A total of 25,952 patient and environmental samples were cultured during the 12-month period of 4/1/76-3/31/77. Serotyping or bacteriophage typing were performed on all strains of Pseudomonas aeruginosa, Serratia marcescens, and Staphylococcus aureus in order to evaluate the epidemiology of these species in cancer patients and their role in possible cross infections. New antibiotics were tested for in vitro minimum inhibitory concentrations, and development of resistance was monitored. A serum bank provides a collection of sera from cancer patients for research purposes.

Project Description: Z01 CM 06027-08 COB

The objective of this project is to perform diagnostic microbiologic investigation of high quality for the study of infection in cancer patients, to provide microbiologic information on the environment of the patients in order to eradicate sources of potential infection, to monitor acquisition of flora of patients to be able to prevent cross-contamination, and to provide microbiologic data for the evaluation of protective environments. This project also provides the microbiologic data for a number of research studies.

The bacteriologic studies were performed by Hazleton Laboratories of America under Contract NO1-CM-67080. Aerobic, anaerobic, and fungal flora were determined on samples; 25,952 examinations were made from 4/1/76-3/31/77. All microorganisms were identified as to genera and whenever possible, to species. For epidemiological purposes, subspeciation of *S. aureus* was determined, and intraspeciation of *S. marcescens* and *P. aeruginosa*, by serotyping. Viral studies were performed bythe Viral Diagnostic Service, Georgetown University, until February 15, 1977; and after that date, by the Diagnostic Laboratory, Microbiological Associates, Walkersville, Md. under Contract NO1-CM-77131.

These contracted facilities provide the microbiologic support for selection of appropriate antibiotic therapy for infection of cancer patients who are receiving therapy for their neoplasms. It also provides the microbiologic support for a number of protocols such as the Pharyngitis protocol, No. 7504; the Herpes zoster protocol, No. 7506; the GVN protocol, No. 7513, etc. as well as other investigations and subsequent publications. It has provided data which allowed the discovery and subsequent elimination of pathogenic fungal spores in the insulating material on the overhead beams in the Cancer Center (Project No. 06264). All of the microbiologic data is stored in computers and is retrievable for investigational purposes (See Project No. 06039).

A serum bank provides a collection of sera from cancer patients for research purposes. This sera is collected on a regular basis from patients at the Baltimore Cancer Research Center. Pertinent data consisting of the patient's name, hospital number, diagnosis, and the dates and amounts of sera collected have been entered into a computer which provides rapid, accurate, and easy accessibility to the sera as to storage, location, and quantity of serum available. These sera are used for a number of research studies by the BCRC Staff, for serologic response to infection by the Microbiology Section, and for collaborative studies with other institutions.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 06039-08 COB

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)
Computerized Microbiology Reporting and Data Retrieval Systems

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Viola Mae Young, Ph.D.	Head, Microbiology Section	CL NCI
Others:	John W. Gerster	Computer Specialist	CL NCI
	William L. Robinson	Computer Specialist	CL NCI
	Richard K.C. Hsieh	Head, HSR	HSR NCI

COOPERATING UNITS (if any)
Diagnostic Laboratory, Microbiological Associates, Walkersville, Md.
Health Services Research, Public Health Service Hospital, Baltimore, Md.
Hazleton Laboratories, Vienna, Va.; Tymshare, Inc., Baltimore, Md.

LAB/BRANCH
Clinical Oncology

SECTION
Microbiology

INSTITUTE AND LOCATION
NCI, NIH Baltimore, Md. 21201

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2/3	1/2	1/6

CHECK APPROPRIATE BOX(ES)
☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline ke

This project is designed to provide a rapid and accurate means of reporting and updating preliminary results of bacterial, fungal, and viral cultures which are performed on samples from cancer patients. The bacterial and fungal cultures are performed at Hazleton Laboratories, Vienna, Va., and the viral cultures at Microbiological Associates, Walkersville, Md. The computer generates a final report and permanently stores the data. Error detection and quality control systems are utilized as a control for the microbiologic data. Summary reports are regularly obtained from the computer and placed in the patients' charts. Retrieval programs are utilized to review data for a number of purposes, particularly for support of patient care and for research purposes. Antibiotic susceptibilities are regularly reviewed for development of resistance to antibiotics in current use. Normal flora and acquired flora of cancer patients have been reviewed through use of computer facilities. Prevalence of various organisms is also reviewed and their predominance in a variety of patient sources: i.e., blood, urine, wounds, etc. Improvements in coding systems have allowed evaluation of media for recovery of microorganisms.

PHS-6040
(Rev. 10-76)

The goal of this project is to provide a rapid and accurate means of communicating results of bacterial, viral, and fungal cultures in cancer patients from the distantly located contracting laboratories to the Baltimore Cancer Research Center. Bacterial and fungal cultures are performed at the Hazleton Laboratories, Vienna, Va., and preliminary information is reported to BCRC within 24 hours. Further cultural data are forwarded as warranted until the work has been completed. A complete report of culture results is then sent to the computer center where the data are passed through an error detection system and the final report generated. The data from the final reports are permanently stored and used as the data base for retrieval purposes. Viral cultures and serological analyses of patients' sera, which are performed at Microbiological Associates, Walkersville, Md., are similarly reported; their data are also stored and used for retrieval purposes.

The numerous computer programs allow retrieval of the data for use by the BCRC staff. Antibiotic susceptibilities of microorganisms are retrieved and reviewed regularly in order that development of resistance to currently used antibiotics can be monitored.

Recent examples of the use of retrievals were the studies of normal flora of cancer patients and of patients with acute non-lymphocytic leukemia in particular, a comparison of skin flora of cancer patients housed at two different locales, a comparison of the recovery of yeast-like fungi from various blood culture media to determine the most efficacious media combination for this recovery, a review of the acquisition of Staphylococcus aureus based on phage types, etc. The microbiologic data are also retrieved in conjunction with the computerized Census Program (Report 06220) which records patient location and attending physician, and checks for inaccuracies between hospital numbers and patient names. The data base is also frequently utilized by the Infection Research Section for information and research pertinent to their studies on patient infection and therapy such as the possible transmission of bacteria between patients who share rooms or have other contacts.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 06219-08 COB

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Studies on Cancer Patient Microbial Flora: Acquisition, Colonization and Infection

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Viola Mae Young, Ph.D.	Head, Microbiology	CL NCI
Others:	Marcia R. Moody	Research Microbiologist	CL NCI
	Maureen J. Morris	Laboratory Scientist	CL NCI
	George Fukui, Ph.D.	Director, Microbiology	Hazleton Labs
	Philip Hartman, Ph.D.	Prof. of Biology	Johns Hopkins Univ.

COOPERATING UNITS (if any)
Hazleton Laboratories, Vienna, Va.
Dept. of Biology, Johns Hopkins University, Homewood Campus, Balto, Md.
Section of Infection Research, COB, BCRC

LAB/BRANCH
Clinical Oncology

SECTION
Microbiology

INSTITUTE AND LOCATION
NCI, NIH Baltimore, Md. 21201

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1-5/6	1-1/2	1/3

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline ke

This project is an ongoing study of the microbial flora of cancer patients. Surveillance cultures which monitor the flora allow detection of endemic or epidemic strains, acquisition of strains, organisms that are unusually resistant, etc. All Serratia marcescens are serotyped and all staphylococci are phage typed. A study of the antibiotic susceptibilities of 2500 bacterial isolates to 4 new aminoglycosides and 2 new penicillins was made. Serratia was the only species to show complete resistance. A retrospective study of 139,768 surveillance cultures to determine cancer patient microbial flora revealed an increase in numbers of Enterobacteriaceae in the upper respiratory tract and skin. Candida spp. were most common in the oral cavity of patients with ANLL (28%). Surveillance cultures and minimum inhibitory concentrations of antibiotics for infecting organisms were performed on patients who were placed on protocols which require this information. Surveillance culture monitoring aids in selection of empiric antibiotics, recognizing the need for isolation or other protective measures, and aids in the design of ongoing infection control procedures.

Project Description:

The objective of this project is an ongoing study of the microbial flora of cancer patients. Routine surveillance cultures of all patients are taken to determine the flora on entrance to the hospital and are repeated on high risk patients throughout the hospital stay to determine acquisition of flora from the hospital environment. The data thus obtained allows early detection of endemic and epidemic bacterial strains and strains with unusual antibiotic resistance so that isolation procedures can be instituted. Unusual organisms that are recovered are studied extensively.

Serotyping of 246 isolates of *Serratia marcescens* revealed no major changes in somatic serotypes. Some strains of *Serratia* showed phase variation of the flagellar antigen (Project No. 06254).

A review of the susceptibilities of 2500 bacterial isolates (21 species) to the aminoglycosides; netilmicin, amikacin, tobramycin, gentamicin, and the penicillins; ticarcillin and carbenicillin, revealed that *S. marcescens* showed the most marked tendency to develop complete resistance to these antibiotics. The majority of the strains were resistant to the penicillins and had a range of 51% to 88% susceptibility to amikacin. Although netilmicin was used for only 8 of the 16 months of this study, 28% of the *Pseudomonas aeruginosa* developed resistance to it; the next highest rate was 17% resistance to gentamicin. *Escherichia coli*, *Citrobacter* spp., and *Enterobacter* spp. developed little resistance (> 71% sensitive); and *Proteus* spp. and *Staphylococcus aureus* even less (>83% sensitive). Conversely, *Klebsiella* spp. showed marked resistance to carbenicillin (3% sensitive) although susceptibility to ticarcillin remained higher (>68% sensitive). With few exceptions, resistant strains have not proved to be a major problem.

A retrospective study of 139,768 surveillance cultures taken from patients at the BCRC between January, 1968 and August, 1975 was undertaken to delineate cancer patient microbial flora. Although, as anticipated, gram positive cocci constitute a major portion of the flora of the upper respiratory tract of the cancer patient, an increase in number of the *Enterobacteriaceae* over those present in healthy adults was found. Skin cultures reflected the same trend (axilla, 11.9%; hand, 7.0%; foot, 6.3%, and perineum, 22.5%). Vaginal cultures yielded 32.5% *Enterobacteriaceae*. Patients who received oral nonabsorbable antibiotics to repress enteric flora and whose rectal and stool cultures were negative, frequently continued to harbor *Enterobacteriaceae* on the adjacent skin. The oral cavity harbored *P. aeruginosa* frequently, as did the stool, except for ANLL patients who had received no therapy; in these patients, *P. aeruginosa* was over 3 times more frequently present in rectal and gingival cultures. Oral *Candida* spp. ranged in frequency of recovery from 12.6% to 17.7% in the cancer patients as a whole, but was present in 28% of cultures from ANLL patients. It is evident that those organisms which are opportunistic pathogens in cancer patients colonize body sites that are not their usual habitat and through aspiration or other methods, provide a means of infection.

Patients who are placed on Protocols No. 7603 and No. 7604, which are designed to evaluate antibiotics, are monitored by surveillance cultures to determine effectiveness of the antibiotics under study. Minimum inhibitory concentrations are performed on the infecting organisms recovered from patients on these protocols to correlate with effectiveness of therapy. Some of the antibiotics are relatively new, and such data must be generated as part of the information necessary to evaluate the drug.

All *Staphylococcus aureus* which are recovered are phage typed and the types entered into a computer (Project No. 06039). Programs have been written which interact with the Census program (Project No. 06220) and which allow comparisons of phage types of S. aureus carried by patients who share rooms, have the same physicians or nursing personnel, etc.

Publications:

1. Schimpff, S.C., Landesman, S., Hahn, D.M., Standiford, H.C., Fortner, C.L., Young, V.M., and Wiernik, P.H. Ticarcillin in Combination with Cephalothin or Gentamicin as Empiric Antibiotic Therapy in Granulocytopenic Cancer Patients. Antimicrobial Agents & Chemotherapy. 10:837-844, 1976.

2. Aisner, J., Schimpff, S.C., Sutherland, J.C., Young, V.M., and Wiernik, P.H. Torulopsis glabrata Infections in Patients with Cancer. Am. Journal of Medicine, 61:23-28, 1976.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 06223-08 COB

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Studies on Pseudomonas aeruginosa in a Cancer Research Center

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Marcia R. Moody, M.S.	Research Microbiologist	CL NCI
Other:	Viola Mae Young, Ph.D.	Head, Microbiology Section	CL NCI
	Maureen J. Morris	Laboratory Scientist	CL NCI
	Norman R. Friedman	Microbiologist	CL NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH
Clinical Oncology

SECTION
Microbiology

INSTITUTE AND LOCATION
NCI, NIH Baltimore, MD 21201

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1-1/6	1	1/6

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline ke

The influence of intrinsic and extrinsic factors, such as antibiotic resistance, genetic characteristics, host immune response, on the incidence and pathogenicity of P. aeruginosa in cancer patients is the prime objective of this study. Preliminary data on antibiotic resistance transfer suggest that nonconjugative plasmids may be responsible for the resistance patterns in various strains of Pseudomonas spp. recovered from sink drains.

In C_3H adult female mice, IgM is the Ig class primarily responsible for protection during the first 2 weeks after immunization, whereas nonagglutinating IgG is responsible for later protection; both are opsonins. Importance of humoral response in the control of P. aeruginosa infection is an ongoing study. The kinetics of Ig class specific antibiody to P. aeruginosa in sera of cancer patients will be studied.

Intraspecies differentiation of Pseudomonas spp. other than P. aeruginosa by antisera is under investigation.

PHS-6040
(Rev. 10-76)

Project Description:

Pseudomonas aeruginosa continues to be an important pathogen in cancer patients and prevention of these infections as well as control of established infections in this susceptible patient population has become essential. This study is structured on an ongoing basis to the incidence of P. aeruginosa in the cancer patient population as well as to the infectious potential of other Pseudomonas spp. The effect of intrinsic and extrinsic factors on the epidemiology of P. aeruginosa, its susceptibility to antibiotics and the host immune response to this bacterium are investigated to provide understanding of mechanisms by which P. aeruginosa can be eliminated or controlled.

The serotypes of P. aeruginosa in cancer patients and their hospital environs are evaluated on a routine basis. In order to establish the serotypic identity of an isolate, three different sets of sera were used and evaluated; similar results were obtained with each set. Currently the identity of the isolate must be confirmed as identical in at least two sets. Techniques that will facilitate the serotyping of rough isolates are under investigation.

Epidemiological monitoring of Pseudomonas spp. other than P. aeruginosa is under study. An identification schema developed by M.R. Moody has facilitated accuracy in speciation. Further intraspecies differentiation of these species by antisera is being investigated.

Phenotypic and genotypic changes in P. aeruginosa and other Pseudomonas spp. are monitored to determine whether 1) epidemiological relatedness of antibiotic resistance is important in the resistance found in this bacterial population, 2) the serotypic identities of strains are influenced by these changes, and 3) these changes have any effect on the virulence of these bacteria. Certain strains of P. putida, P. maltophilia, and P. aeruginosa that had been recovered concurrently from sink drains in patients' rooms were found to be resistant to the aminoglycosides, carbenicillin, chloramphenicol, and the cephalosporins. This resistance was not transmissible to a susceptible recipient in R factor transfer studies. These preliminary data suggest that nonconjugative plasmids may be involved. Spontaneous curing of resistance to gentamicin and carbenicillin occurred in various strains of P. aeruginosa but no discernible pattern among the strains cured was evident. Investigative studies of pseudomonas genetics will continue.

C_3H adult female mice were used as experimental models in the investigation of Ab synthesis against P. aeruginosa in the immune host. The immunogen was a P. aeruginosa type 2 lipopolysaccharide fraction from Parke-Davis and Co. All mice were immunized with 100 µg of the fraction, and booster doses of 10 µg and 1 µg of the fraction were given to a selected group of mice 15 weeks after 1^{o} immunization. The sequence of P. aeruginosa antibody production in the serum after primary and secondary stimuli was examined by a specific immunoglobulin assay in which the sensitivity of detection was increased by specific anti IgG and anti IgM globulin reagents. Multiple serum samples from immunized and nonimmunized mice were examined to detect the class of Ig present in the serum; a selected group of mice were challenged with various LD_{50} doses at the time of test bleeds in order to determine the effectiveness of these antibodies in protecting the mice against lethal challenges. Passive Ab transfer protection experiments were also performed to test the protective ability of the Ab in these sequential sera. Ab was present up to day 14. After this time, the titer level of agglutinating Ab declined while the titer level of the nonagglutinating Ab increased.

Nonagglutinating IgM levels, while higher than the agglutinating Ab levels, had the same slope of decline as the latter levels in that the level declined as the post 1° immunization time increased. In contrast, nonagglutinating IgG levels increased so that by week 14, post immunization, no detectable agglutinating Ab was present, but IgG was present at a 1:160 dilution. The antibody response to 2° immunization began to increase sooner than in the 1° immunization and the response was dose related in that higher levels were obtained with the larger booster dose. Nonagglutinating Ab was still IgG predominantly and was 32-fold higher than the agglutinating titer.

Nonagglutinating immune Ab was as effective in the protection against challenge doses as the agglutinating; immunized mice were protected against 1 LD_{50}, 10 LD_{50}, and 100 LD_{50} doses when only agglutinating Ab was detectable (2 wks) and when only nonagglutinating Ab was detectable (14 wks). Protection tended to be dose related as the larger the booster dose, the better the protection; however, mice that were only 1° immunized were still protected against challenge doses at 33 weeks post 1° immunization. Protection could also be transferred to nonimmunized mice by the injection of the various serum samples into the peritoneal cavity 2 hours prior to challenge.

Bactericidal and osponophagocytic tests were performed to determine the mechanism by which the Ab protects against challenge dose. The protective Ab was found to be an opsonin as it was shown that P. aeruginosa was killed by the immune serum Ab only in the presence of cells and complement. However, there was not an appreciable difference between the opsonophagocytic index of mice 2° immunized with 10 μg or 1 μg.

A study of Ig class specific antibody to P. aeruginosa in the sera of cancer patients is now in progress.

Publications:

1. Moody, M.R. "Effect of Acquisition on the Incidence of Pseudomonas aeruginosa in Hospitalized Patients", Pseudomonas Aeruginosa, Ecological Aspects and Patient Colonization, pp. 111-132, Raven Press, New York, N.Y., 1977.

2. Young, V.M. (Ed.), Pseudomonas Aeruginosa, Ecological Aspects and Patient Colonization, "Preface" p. v-vi and "Introduction" p. xi-svii. Raven Press, New York, N.Y., 1977.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 06254-08 COB

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Research Activities Directed Toward Improvement of Microbiology Laboratory Techniques

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Viola Mae Young, Ph.D.	Head, Microbiology Section	CL NCI
Others:	Marcia R. Moody	Research Microbiologist	CL NCI
	Maureen J. Morris	Laboratory Scientist	CL NCI
	Norman Friedman	Microbiologist	CL NCI
	Michael T. Hargadon	Microbiologist	Hazleton Labs
	George Fukui, Ph.D.	Director, Microbiology	Hazleton Labs

COOPERATING UNITS (if any)

Hazleton Laboratories, Vienna, Va.
Diagnostic Laboratory, Microbiological Associates, Walkersville, Md.

LAB/BRANCH
Clinical Oncology

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NCI, NIH Baltimore, MD 21201

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
5/6	2/3	1/6

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline k

The continuing objective of this project is the evaluation and implementation of new methodology whenever it offers the advantages of more rapid or precise identification of microorganisms recovered from patient samples or when it results in cost reduction. The cause for weakly positive coagulase tests for Staphylococci that could not be phage typed was determined to be acidity due to incubation in a CO_2 incubator. A new schema for the identification of "non-fermenting gram negative bacilli" has been developed, tested, and adopted for use. The API enteric strip is under evaluation for identification of the Enterobacteriaceae. During serotyping of the Serratia marcescens, it was discovered that the apparent fluctuations in flagellar antigens were due to phase variation. Crytococcal antigens and Toxoplasma antibodies are determined on a routine basis and photomicrographs are made for documentary files. A new system for rapid retrieval of stored stock cultures was devised. A review of blood culture media from which Candida spp. and Torulopsis glabrata were most frequently recovered has led to utilization of several Sabouraud's slants for blood of patients suspect of having fungemia.

Evaluation and implementation of new methodology in microbiology are continuing objectives of this project. These investigations offer advantages for rapid and accurate recovery and identification of microorganisms from clinical specimens obtained from cancer patients. The development and standardization of methods for antibiotic susceptibility, for potentiation effect of chemotherapeutic agents on antibiotics are essential for effective support data to the clinician.

Staphylococcus aureus is still the chief cause of infection in cancer patients. All strains are phage-typed, and as new phages become available, they are also included in the already established phage-typing system. One method that is used routinely in the identification of S. aureus is the coagulase test. Due to an increased number of staphylococcal strains that gave weakly positive coagulase reactions but were nonreactive to S. aureus phages, a study of the plasma used in the test was undertaken. It was found that uninoculated plasma from a major commercial supplier would clot when the pH was lowered. As the tests were routinely incubated in a CO_2 incubator, the CO_2 absorbed by the plasma would lower the pH and a weak clot formation would occur. Coagulase tests are no longer incubated under CO_2 and weakly positive reactions have been eliminated. A plate coagulase test which would decrease the laboratory time needed to identify S. aureus is under investigation.

The genera, Pseudomonas, Alcaligenes, Acinetobacter, and Bordetella, are now speciated by a schema developed at the BCRC. Over 700 isolates were examined by two different laboratories and the results obtained led to identical speciations. Intraspecies differentiation of "non-aeruginosa" pseudomonads with antisera prepared in rabbits is under investigation.

The API enteric strip for the speciation of the Enterobacteriaceae is under study by the Hazleton Laboratories, Vienna, Va. Appropriate isolates will be used to inoculate the conventional media and API strip, and the cost, time, ease of use, etc. will be evaluated to determine the feasibility of API strips for the identification of enteric bacteria.

There has been increasing concern about Serratia marcescens as an infectious agent and its epidemiology in various institutions. Serotyping was undertaken and the predominant 0 groups were found to be 014, 012, 04, and 01. These somatic antigens appeared to be quite stable and there were rarely variations or changes of 0 groups in strains from the same patient. However, flagellar antigens tended to fluctuate between strains in the same 0 groups and from the same patients. Investigations into this so-called "instability" determined that apparent fluctuations were caused by the strains being bi-phasic (in some strains, triphasic); i.e., more than one flagellar antigen exists in the same strain. When such Serratia strains are repeatedly tested, either phase may be manifested. Further studies are in progress.

Tests for the detection of Cryptococcus neoformans antigen in sera and cerebrospinal fluids from cancer patients are now performed on a routine basis at the BCRC and on a consultant basis for specimens submitted by the VA hospital. This test has proved very useful; in one case, the antigen could be detected in the CSF on numerous occasions without concommitant recovery of the organism from the same samples.

A review of patients' blood cultures from which Candida spp. and Torulopsis glabrata were isolated and the media from which the fungi were recovered revealed that this recovery was more frequent from the single Sabouraud slant on which 1 ml of blood was placed than from 3 bottles (5 ml inoculum each) and 1 tube of liquid media (1 ml inoculum). As a consequence, all patients who are suspected of having fungemias of Candida or Torulopsis have blood withdrawn that is used to inoculate several Sabouraud slants to ensure better recovery of this microorganism.

As previous studies have revealed that Aspergillus spp. are frequently present in nasal cultures prior to being infected with these microorganisms and that cultures of the nose before fungi are recovered often yield no bacteria, a study has been set up to look for fungal invasion of the nares in patients who are receiving antibiotics and from whom no bacteria have been recovered from the nasal cultures. Special fungal media (Czapek agar) are utilized to detect the growth of fungi and nasal scrapings taken to be examined microscopically. Although the utilization of Czapek agar has not enhanced the detection of Aspergillus spp., its selectivity for these fungi makes it a useful media for recovery of Aspergillus.

The search for antibodies to Toxoplasma gondii in the sera of cancer patients has been facilitated by the use of a Toxoplasma kit from IBL. This test is now available on a routine basis.

A new method has been devised for the storage of stock cultures of microorganisms recovered from patients. These organisms are often utilized for various research purposes. Retrieval of specific strains has been a problem because strains and species from one individual were kept together and not cataloged. The new, simplified system allows easy retrieval on demand.

A permanent record by means of photomicrographs is now made of all interesting and unusual findings from patient samples. These photomicrographs may be used for teaching purposes or made a part of the patient's permanent record.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 06938-03 COB

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Environmental studies to prevent Hospital Acquisition of Potentially Dangerous Microflora

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Viola Mae Young, Ph.D.	Head, Microbiology	CL NCI
Others:	Norman R. Friedman	Microbiologist	CL NCI
	Maureen J. Morris	Laboratory Scientist	CL NCI
	Marcia R. Moody	Research Microbiologist	CL NCI
	George Fukui, Ph.D.	Director, Microbiology	Hazleton Labs

COOPERATING UNITS (if any)

Hazleton Laboratories, Vienna, Va.
Section of Infection Research, COB, BCRC

LAB/BRANCH
Clinical Oncology

SECTION
Microbiology

INSTITUTE AND LOCATION
NCI, NIH Baltimore, MD 21201

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1-1/6	1	1/6

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline k

The objective of this project is to survey the patients' environment, and the air, food, water, and products which they utilize in order to detect microorganisms potentially pathogenic for such high risk patients. Patients who carry such pathogens are surveyed to determine if the organisms are being shed into the environment. This study of "shedders" has led to routine isolation of mycosis fungoides patients who carry Staphylococcus aureus. Personnel are surveyed to detect carriers of pathogenic bacteria and procedures are instituted to prevent the spread of these organisms. Effective means of elimination of hazardous organisms are sought. Skin disinfectants were evaluated; as a result, personal preference of 2 effective products is allowed. Foods were examined and methods of decontamination of fresh foods are under study. Decontamination procedures for sink drains are being evaluated. Protective environments for high risk patients are also being studied. As a result of intensive efforts to protect the patient, organism acquisition has decreased considerably. Follow-up studies following decontamination procedures to eliminate Aspergillus spores from overhead fire-proofing material revealed very few fungal spores in ambient air.

The objective of this project is to survey the patients' environment, air, food, water, products used in their care, and the personnel responsible for patient care, in order to detect microorganisms that can be potentially pathogenic for the cancer patient. Evaluations of preventive methods are performed on an ongoing basis, and those methods that are effective in the elimination of such organisms are then instituted. Surveillance procedures are utilized routinely for monitoring the environment.

A study has been initiated to detect shedders of potentially pathogenic microorganisms. All patients who have infected wounds, or who are heavily colonized with S. aureus, P. aeruginosa, or with a microorganism that is highly resistant to many antibiotics, could shed such a bacterium into the environment where it could be transmitted to a new susceptible host a a later time. It was discovered that a patient, such as one with mycosis fungoides whose skin is heavily colonized with S. aureus and from whom there is much skin desquamation, will be a heavy shedder of the S. aureus with which he is colonized. Such patients are then placed under appropriate isolation procedures.

Hand carriage of microorganisms from patient to staff to patient or from environment to staff to patient needs to be kept at a minimum as patients at the BCRC are at high risk of infection. Therefore, any product that could limit the number of microorganisms carried on the hands would be useful. One product, an antiseptic foam called "Foamaseptic", was in current use with generally satisfactory results when another antiseptic foam, "Septisol", became available. As complaints of a greasy buildup on the hands after repeated usage of Foamaseptic were frequent, a comparison between the two products was undertaken in order to ascertain if the second foam was as effective as the first in reduction of hand flora, but more acceptable in residual physical properties. It was found that 1) both products were equally effective in reducing hand flora, 2) whereas Foamaseptic caused a greasy buildup on hands, Septisol tended to dry and mildly irritate the skin. As a consequence, both products were made available and their usage determined by the preference of the user.

It had been proven that the source of Aspergillus spp. that were responsible for the increased number of fungal infections among the patients was the fireproofing material on the overhead beams between the true ceiling and the fake ceiling. These beams were sprayed with Copper-8-Quinolinolate to decontaminate them in the whole cancer patient area. A year after the spraying, the area still remained free of Aspergillus spp. when the area was cultured. A controlled series of air samples taken with special fungal media revealed very few fungi in the ambient air. The incidence of Aspergillus infections was reduced to zero during the 12 months subsequent to spraying.

Controlled environments designed to protect patients from infection continue to be evaluated. Weekly cultures of sites in each room have been reduced to 11-13, as analyses of data revealed that results obtained from these sites were as informative as those obtained from a greater number of sites. Air sampling in these rooms has been increased in order to determine the long-term effectiveness of the various air filtration systems in the removal and reduction of bacteria from the air in the rooms. The microbiological data that are obtained from the air samples and cultures of room sites continue to serve as a means of monitoring proper decontamination procedures, microorganism shedding by patients, and the likely sources of organisms that could be acquired by the patients. As a result of the intensive efforts undertaken to prevent the patient from acquisition of potentially pathogenic microorganisms from the hospital and thus prevent hospital-acquired infection, acquisition rates during the hospital stay has dropped. The previous level of acquisition was 8 organisms within 6 weeks; this level is now an average of 2 acquired organisms during the same period of time.

Cooked foods are the only diet given to the patients, as cooked foods are seldom contaminated. Cultures of these foods are done routinely on a scheduled basis in order to determine that they remain free from bacterial contamination. A study has also been initiated to determine the feasibility of adequately decontaminating outside surfaces of uncooked foods, such as watermelons, cucumbers, etc., and of slicing these foods without contamination of the slices so that they could be added to the now very restricted diets of the cancer patient.

Various genera of bacteria; i.e., Pseudomonas, Klebsiella, Enterobacter, etc. are frequently recovered from the sink drains in the patients' rooms. In order to eliminate sites as potential sources of pathogens that could be acquired by patients, techniques by which the drains can be decontaminated are tested as they become available. Two methods, a cut-off valve system, which allows disinfectant longer contact time with the inside drain surface, and an electric coil wrapped drain, which can be heated to a temperature designed to eliminate bacteria through drying of pipe inner surface, are currently under study.

Cancer patients undergoing therapy on an outpatient basis are housed in a nearby motel. Thus far, this environment has proven to be free from potentially dangerous microflora; however, periodic culturing of the rooms is performed in order to ensure that the environment remains pathogen-free.

Publications:

1. Young, V.M., Clinical Gnotobiotics, American Journal of Clinical Nutrition, IN PRESS (Aug. or Sept.), 1977.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 06043-06 COB

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Automated Monitoring of Drug Therapy

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Clarence L. Fortner, Director, Clinical Research Pharmacy Service, CL-NCI

OTHER: Richard Hsieh, Chief, Health Services Research, USPHS Hospital
William R. Grove, Clinical Pharmacist, CL-NCI
Peter H. Wiernik, Chief, Medical Oncology Branch, CL-NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH
Clinical Oncology Branch

SECTION
Clinical Research Pharmacy Service

INSTITUTE AND LOCATION
NCI, NIH, Baltimore, Maryland 21201

TOTAL MANYEARS: 1.75	PROFESSIONAL: .25	OTHER: 1.25

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline ke

The objective of this project is to provide an efficient and rapid method of automated collecting of data concerning the clinical trials of experimental drugs.

The system of computer processing of drug administration information provides accurate periodic summaries of all BCRC patient's drug therapy. Each drug card is typed containing the name of the drug, dose, route of administration, frequency, date, and patients name and medical record number. Each card also contains the direct administration record as charted by the nurses at the time of administration. These cards are processed by optical character recognition and produce a weekly summary of all drugs ordered and their administration record, organized by patient and by problem oriented system.

This system fulfills all the requirements of drug ordering, administration and recording in addition to providing machine-readable data for computer input. This system eliminates the transcription operations usually associated with preparation of input for computers, and provides a data pool for clinical studies.

The objective of this project is to provide an efficient and rapid method of collecting data concerning the clinical trials of experimental drugs.

This is being studied by a computerized method of ordering and recording all drug therapy.

A system of processing drug administration information provides accurate, weekly summaries of each patient's complete drug therapy. The physician writes each drug order directly on a medication card, producing four copies: the medicine card copy, the pharmacy copy (for entry to a patient drug profile), and copies for medical records and automated analysis. The nurse charts medications given directly on the first copy of the drug form at the time of drug administration. This data is read weekly by optical character recognition. The data is collated and patient drug summaries are printed listing drug, dose, total dose, frequency of administration and a daily record of administration or omission of each drug. A monthly drug utilization report is also obtained as a by-product.

The problem number and statement according to the Weed's problem oriented health record system has been incorporated into the form which allows each drug to be listed by problem number on the weekly drug summary for each patient. Space on the drug form has also been allotted for the patient's height, weight, and body surface area. This gives the pharmacist enough data to recalculate all drug doses listed in research protocols. Not only does this recalculation protect the patient, but it also protects the research environment by insuring the accuracy of all doses administered. This system fulfills all the requirements of drug ordering, administration and recording, in addition to providing machine-readable data for computer input. The summaries are checked by the pharmacists, and become a permanent record of drug therapy both in the patient's chart and in the pharmacy. This system eliminates the transcription operations usually associated with preparation of input for computers, and provides a data pool for clinical studies, analyzing drug effect, toxicity, and drug interaction. This system has reduced errors in drug administration and has increased staff awareness of the problems inherent in quality drug administration. A complete list of all drugs administered to the patient are recorded by problem numbers in a concise and orderly fashion in the patient chart. This list of all drugs administered has been used to obtain information about the cost of drug treatment for single medical problems. This cost may be found because of the development of a new cost model referred to as Problem Oriented List of Charges (POLC). This model is directly related to the Problem Oriented list of all drugs administered to each patient described above. The POLC may be used to determine the drug cost for single problems such as fever, granulocytopenia, etc. This system, as outlined, has provided a more comprehensive and efficient record of cancer chemotherapy drug trials.

Publications:

Esterhay, R.J., Vogel,V.G., Fortner,C.L., Sharpiro,H.M., Wiernik,P.H.; Cost Analysis of Leukemia Treatment- A Problem Oriented Approach, Cancer 37: 646-652, 1976.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 06236-06 COB

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Role of the Pharmacist in Obtaining a Patient's Drug and Allergy History

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Clarence L. Fortner, Director, Clinical REsearch Pharmacy Service,CL-NCI

OTHER: William R. Grove, Clinical Pharmacist, CL-NCI
John F. Bender, Clinical Pharmacist, CL-NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH
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Clinical Research Pharmacy Service

INSTITUTE AND LOCATION
NCI, NIH, Baltimore, Maryland 21201

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.2	0.2	0

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☐ (a1) MINORS ☑ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline k

The purpose of this study is to evaluate the feasibility of having a pharmacist interview all patients referred to the BCRC to obtain a complete drug and allergy history.

Three major areas of medication history are investigated: prescription medications, over-the-counter (OTC) medications, and previous allergic reactions. The original copy of the interview report is placed in the history section of the patient's hospital chart. Duplicate copies are retained in the patient's drug profiles in the pharmacy. The pharmacist is responsible for placing all known allergic reaction information on the problem sheet of the patient's chart. All medications brought to the hospital by the patient are obtained at the time of the interview, and are stored in the pharmacy during the period of hospitalization.

In addition, new outpatients to BCRC are also interviewed by the pharmacist as they receive their first outpatient prescriptions.

These interviews have provided the pharmacist with patient contact at the time of admission which has increased the pharmacists awareness of patient care.

These interviews have demonstrated that the pharmacist can provide an additional useful service by obtaining a complete drug and allergy history information.

PHS-6040
(Rev. 10-76)

The purpose of this study is to evaluate the feasibility of having a pharmacist interview all patients referred to the BCRC to obtain a complete drug and allergy history.

A guideline for the interview has been developed which investigates three major areas: prescription drugs, over-the-counter (OTC) drugs and allergy/ sensitivity history. All pertinent information, particularly regarding allergy history, is brought to the direct attention of the physician. The pharmacist is also responsible for placing all known allergic reaction information on the problem sheet of the patient's chart and on the front of the patient's Kardex. The information obtained from the interview is placed in the history section of the patients chart. Duplicate copies are retained in the patient's drug profiles in the pharmacy.

All medications brought to the hospital by the patient are obtained at the time of the interview, and are stored in the pharmacy during the period of hospitalization.

During the last year, the outpatient pharmacist has begun obtaining the same drug and allergy history information on new outpatients to BCRC, at the time they receive their initial prescriptions.

These interviews have provided the pharmacist with patient contact at the time the patient initially enters the BCRC program and has increased the pharmacist's awareness of patient care. These interviews have demonstrated that the pharmacist is interested in patient care, and that he can provide an additional useful service.

The drug and allergy interview by the pharmacist has become an integral part of the clinical program. The present study will be continued.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 06237-07 COB

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Development of a Clinical Research Pharmacy Service

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Clarence L. Fortner, Director, Clinical Research Pharmacy Service, CL-NCI

OTHER: William R. Grove, Clinical Pharmacist, CL-NCI
Robert Foreman, Clinical Pharmacist, CL-NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH
Clinical Oncology Branch

SECTION
Clinical Research Pharmacy Service

INSTITUTE AND LOCATION
NCI, NIH, Baltimore, Maryland 21201

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☑ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline k

The objective of this study is to determine the role of the pharmacist in the patient care area, and as a member of the health care team actively contributing to the patient's care.

The pharmacist has proven that he can be patient-oriented rather than product-oriented. The pharmacist utilizing new methods has become a valuable member of the health care team and in contributing directly to the patient care. The following has been accomplished: improving drug prescribing practices by promoting the rational and safe use of drugs, preventing potential drug-drug interactions, detecting and preventing adverse drug reactions, detecting drug induced laboratory test abnormalities, detecting possible drug induced disease, providing drug information, continuing education for various members of the health care team, counseling the patient on proper use of drugs, and detecting and preventing intravenous admixture incompatibilities.

PHS-6040
(Rev. 10-76)

The objective of this study is to determine the role of the pharmacist in the patient care area, and as a member of the health care team actively contributing to the patient's care. Patient education has been added to this study. The objective of this education will be to increase compliance and reduce problems for outpatients receiving drugs.

The pharmacist is located in the midst of the patient care area in order to allow him to investigate new concepts, approaches, and research opportunities to aid in the patient care. The pharmacist attends morning and evening ward rounds, reviews charts, interviews patients and is familiar with the patients' condition. Pharmacy rounds are convened immediately after the morning medical rounds. All pharmacists attend, as all new problems are reviewed. This provides the pharmacists with all of the patient current problems. Therefore he can intelligently discuss therapy problems with the patients's physician during the working day. With this information, he can also provide the staff with unsolicited information.

An integral part of this service is the therapy surveillance system. The nucleus of the drug surveillance system is the individual patient drug profile. The pharmacist receives a direct copy of the physician drug order and transcribes it to the patient drug profile. The drug profile includes the patient name, primary and secondary problems, physician name, known allergies or idiosyncrasies and all drug orders. The pharmacist reviews each new drug order for completeness and recalculates all protocol drug dosages then reviews the drug profile against the new order for drug interactions, laboratory problems, dietary problems and possible adverse drug reactions. The drugs are then dispensed through a unit dose system which provides accurate records. All returned doses to the pharmacy are investigated for reason of non-compliance. The monitoring system involves evaluating the necessity of continued medication orders.

The pharmacist has shown that he can be patient oriented rather than product oriented. The pharmacist utilizing the above methods has become a valuable member of the health care team and contributes directly to the patient care. The following has been accomplished: improving drug prescribing practices by promoting the safe and rational use of drugs, preventing potential drug-drug interactions, detecting and preventing adverse drug reactions, detecting drug induced laboratory test abnormalities, detecting possible drug-induced diseases, providing drug information, counseling the patient on proper use of drugs, and detecting and preventing intravenous admixture incompatibilities. This has reduced drug exposure to the patient, decreased nursing and pharmacy time spent administering and preparing drugs and reduced cost.

A higher quality of drug prescribing and reduced medication errors has contributed toward better patient care and has enhanced the quality of the research data.

Drug information is becoming a major by-product of locating the pharmacist in the patient care area. This information is being requested by all members of the health care team. Continuing education is also involved in this process of providing drug information. The impact of this service will continue to be studied by the audit process.

The pharmacist is making rounds with a nurse to improve compliance of patient taking non-absorbable oral antibiotics and other medications.A pharmacist is doing audiograms of patients receiving systemic aminoglycosides.

Present studies will be continued and additional methods to improve patient care, will be further researched.

PUBLICATIONS:

Grove,W.R., Fortner,C.L.,; Carcinoma of the Breast; Journal of the American Pharmaceutical Association Vol. NSC16, No. 5:254-262, 1976

Grove,W.R., Bender,J., Fortner,C.L.,; A Benzquinamide Induced Extrapyramidal Reaction: Case Study; Drug Intelligence and Clinical Pharmacy Vol. 10: 638-639 1976

Schimpff,S.C., Landesman,F., Hahn,D., Standford,H., Fortner, C.L., Young,V., Wiernik,P.H.,; Ticarcillin in Combination With Cephalothin or Gentamicin as Empiric Antibiotic Therapy in Granulocytopenic Cancer Patients; Antimicrobial Agents and Chemotherapy, 837-844, 1976

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 06912-03 COB

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Antineoplastic Drug Displays for the Computerized Problem-Oriented Medical Information System

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: William R. Grove, Clinical Pharmacist, CL-NCI
Clarence L. Fortner, Director, Clinical Research Pharm. Service, CL-NCI

OTHER: Paul Chang,M.D., Senior Investigator Med. Oncology Service, CL-NCI
Robert Esterhay, M.D., Senior Investigator, CL-NCI
Peter Walton, M.D., PROMIS, Laboratory, Dept. Med., University of Vermont, Burlington, Vermont
Peter Wiernik, M.D., Chief, Medical Oncology Service

COOPERATING UNITS (if any)
PROMIS Laboratory, Dept. of Medicine, University of Vermont

LAB/BRANCH
Clinical Oncology Branch

SECTION
Clinical Research Pharmacy Service

INSTITUTE AND LOCATION
NCI, NIH, Baltimore, Maryland 21201

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.1	.1	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline ke

The objective of this project is to prepare referenced summarized pharmacologic information which is readily retrievable at the touch-sensitive cathode ray screen terminal located at the BCRC nursing station.

Current literature is reviewed to obtain accurate and complete drug information on antineoplastic agents. The standardized format consists of separate display pages for information as follows: DRUG NAME, CHECK PROBLEM LIST FOR, SIDE EFFECTS TO WATCH FOR, DRUG INTERACTIONS, TEST INTERFERENCES, USUAL DOSAGE, PHARMACEUTICAL DATA, MECHANISM OF ACTION, AND METABOLISM AND EXCRETION. Reference codes are included with all pages of information, which may be further explored by contacting either the Clinical Research Pharmacy Service of the BCRC or the PROMIS Laboratory, University of Vermont. After completion, display sequences are sent to the PROMIS laboratory to be audited for accuracy, completeness, and correctness of clinical judgement before being entered into the computerized system.

The completed antineoplastic drug displays provide ready access to referenced summaries of the current medical literature. They form an integral part of the contribution to improved patient care and improved medical records played by the PROMIS system.

PHS-6040
(Rev. 10-76)

The objectives of this project are to prepare referenced summarized pharmacologic information which is readily retrievable at the touch-sensitive cathode ray screen terminal located at the BCRC nursing station. These displays are to be complete, concise and accurate summaries of currently-available literature. Additional antineoplastic drugs are added to the computerized drug displays already on file with the PROMIS system, Burlington, Vermont.

Current literature is reviewed to obtain accurate and complete drug information on antineoplastic agents. This information is then organized to a specific format to conform with the over 20,000 displays currently included in the PROMIS system. The standardized format consists of separate display pages for information as follows: DRUG NAME, CHECK PROBLEM LIST FOR, SIDE EFFECTS TO WATCH FOR, DRUG INTERACTIONS, TEST INTERFERENCES, USUAL DOSAGE, PHARMACEUTICAL DATA, MECHANISM OF ACTION, AND METABOLISM AND EXCRETION. Reference codes are included with all pages of information, which may be further explored by contacting either the Clinical Research Pharmacy Service of the BCRC or the PROMIS Laboratory, University of Vermont. After completion, display sequences are sent to the PROMIS Laboratory to be audited for accuracy, completeness, and correctness of clinical judgement before being entered into the computerized system. Previously entered data may be updated as necessary by providing the new information and references to the PROMIS laboratory for incorporation.

The completed antineoplastic drug displays provide ready access to referenced summaries of the current medical literature. They form an integral part of the contribution to improved patient care and improved medical records played by the PROMIS SYSTEM.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 06937-02 COB

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

A Patient Oriented Outpatient Drug Distribution Service

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Clarence L. Fortner, Director, Clinical Research Pharmacy Service, CL-NCI

OTHER: William R. Grove, Clinical Pharmacist, CL-NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH
Clinical Branch

SECTION
Clinical Research Pharmacy Service

INSTITUTE AND LOCATION
NCI, NIH, Baltimore, Maryland 21201

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.2	1.2	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☑ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline k

The objectives of this project are to provide optimal patient care through the pharmacist's use and maintenance of an individual outpatient drug profile to provide necessary drug information and administration instructions to outpatients, and to dispense prescriptions to outpatients.

When the pharmacist receives the prescriptions from an outpatient, he reviews them for completeness and against any previously prescribed drugs for drug-drug interactions and any known allergies, and then adds the new prescription information to the outpatient drug profile. When a patient is admitted or discharged, the outpatient profile serves as a method by which the pharmacist may verify that necessary medications are continued. The prescriptions will then be prepared and dispensed, with the pharmacist advising the patient of common side effects and specific administration instructions. Patient compliance is stressed by the pharmacist.

PHS-6040
(Rev. 10-76)

The objectives of this project are to provide optimal patient care through the pharmacist's use and maintenance of an individual outpatient drug profile, to provide necessary drug information and administration instructions to outpatients, and to dispense prescriptions to outpatients. These studies will include a method of best detecting drug-drug interactions, drug-food interactions and known patient allergies prior to dispensing the drug to the patient. Also the most appropriate and efficient method of utilizing the outpatient drug profile is to be determined.

New patients will be interviewed by the pharmacist to determine prior drug usage and any known allergies or sensitivities. Any relevant information found is included in the appropriate area of the outpatient drug profile as well as brought to the attention of the physician.

When a prescription is received by the pharmacist, he reviews it for completeness, rational drug therapy, and against the outpatient drug profile for drug-drug interactions and allergy information. Any changes in therapy particularly regarding antineoplastic therapy, are verified with the physician. Several inadvertant changes with potentially serious consequences have been averted by this method. In addition, any situations where prescriptions are not written that should have been, particularly with chronic medications, are investigated.

When a patient is admitted or discharged, the outpatient drug profile serves as a method by which the pharmacist may verify that necessary medications are continued.

The outpatient profile is useful in providing answers to questions from physicians concerning previous outpatient medications.

The patient will be advised by the pharmacist, at the time of dispensing, of common side effects, drug-drug interactions, drug-food interactions, and specific administration instructions, to ensure optimal effectiveness from the medications. Reference standards for patient consultation are being prepared by the pharmacist, particularly in the area of antineoplastic agents. The importance of patient compliance is stressed by the pharmacist. When follow-up instructions indicate poor patient compliance, the pharmacist promptly notifies the physician.

Results indicate proper use of this individual outpatient profile increases proper utilization of outpatient prescriptions and increased patient compliance.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 06238-06 COB

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)
Development of a Unit Dose Service and the Preparation of Parenteral Drugs

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Clarence L. Fortner, Director, Clinical Research Pharmacy Service,CL-NCI

OTHER:William R. Grove, Clinical Pharmacist, CL-NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH
Clinical Oncology Branch

SECTION
Clinical Research Pharmacy Service

INSTITUTE AND LOCATION
NCI, NIH, Baltimore, Maryland 21201

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
4	2	2

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☑ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline k

To improve patient care through a new drug delivery system and to provide more accurate information regarding the disposition of drugs.

The two systems, unit-dose and I.V. admixture, have proven to be advantageous over previous drug distribution systems. They have placed the drug preparation and distribution in the hands of the pharmacist. These systems have accomplished the following: reduced medication errors, reduced medication cost, enhanced the pharmacist as a drug information consultant by relating him more closely to the patient, reduced the possibility of contamination of the drug, permits nurses' to use time more effectively in direct patient care, and in general allows the hospital to demonstrate control over the distribution of all drugs. In addition, the pharmacist has instituted record systems that have improved retrieval of the patient's drug records.

The objective is to improve patient care through a new drug delivery system and to provide more accurate information regarding the disposition of drugs.

A unit dose of medicine is a single dose of medicine packaged for an individual patient. This single dose package is labeled with a name, strength, and control number of the drug, and it is ready to be administered to the patient. These drugs are ordered by the physician and they are placed in the individual patients drawer of a medication cart by the pharmacist. This cart is designed so that there is a single drawer for each patient's drug. All of the parenteral products are prepared aseptically in a laminar air flow enviroment, and are dispensed in a clearly labeled ready to use form. Parenteral products are prepared for not more than a 24 hour period to ensure full potency. The parenteral drugs are not placed in this cart. They are carried directly to a refrigerator in the nursing unit. Control procedures for checking the composition of the product prepared, and for monitoring bacterial and particulate matter contamination of these preparations have been instituted. Pertinent information regarding the drugs dispensed is recorded on specially designed forms that are filed according to the patient's name. This information is also recorded by drug and lot number for each investigational agent.

The two systems, unit-dose and I.V. admixture, have proven to be advantageous over the old ward stock system of drug distribution. They have placed the preparation and distribution in the hands of the pharmacist. These systems have accomplished the following: reduced medication errors, reduced medication cost, enhanced the pharmacist as a drug information consultant by relating him more closely to the patient, reduced the possibility of contamination of the drug, permits nurses to use time more effectively in direct control over the distribution of all drugs. In addition, the pharmacist has instituted record systems that have improved retrieval of the patient's drug record. Physicians utilized these records daily in ordering medications for patients and have found them to be a useful and accurate source of information.

During the past 15 months the pharmacy service has dispensed approximately 254,743 oral doses of medication to inpatients, 31,125 I.V. admixtures, 13,475 doses of parenteral investigational cancer chemotherapeutic agents, and 3,342 sundry sterile preparations.

By reducing medication errors , and improving the accuracy of the records of disposition of cancer chemotherapeutic agents and the other unit dose drugs the quality of patients care has increased. These delivery systems have been integrated with the development of the Clinical Research Pharmacy Service which is providing the patients with better care. Thus, the research drug data will be improved.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 06943-02 COB

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Audit of the Outpatient Drug Profile

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	William R. Grove	Clinical Pharmacist CL-NCI
OTHER:	Clarence L. Fortner	Director, Clinical Research Pharmacy Service CL-NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH
Clinical Oncology Branch

SECTION
Clinical Research Pharmacy Service

INSTITUTE AND LOCATION
NCI, NIH, Baltimore, Maryland 21201

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.1	.1	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☑ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline ke

The purpose of this study is to promote optimal patient care through appropriate utilization of the outpatient drug profile. The process audited is the pharmacist's completion and utilization of the outpatient drug profile. The audit criteria previously defined as audit standard, method, interval, audit form, and feedback system were used.

A second audit of fifteen randomly selected outpatient profiles was performed. Several observations were made, including: Prescription entries in the profile are being completed appropriately in 90% of the instances; no significant drug-drug interactions were dispensed; no drugs were dispensed in violation of any known allergies or sensitivities; all prescriptions written and filled were within the boundaries of rational drug therapy; 93% of drugs written on research protocols were recorded in the appropriate log records; 69% of the prescriptions were dispensed with an indication if a consultation was performed by the pharmacist; problem numbers were indicated on 89% of prescriptions written.

Several recommendations were made to the Clinical Research Pharmacy Service staff, based upon the results of this audit, to improve the utilization of the outpatient drug profile.

PHS-6040
(Rev. 10-76)

The purpose of this study is to promote optimal patient care through appropriate utilization of the outpatient drug profile.

Outpatient drug profiles are randomly selected and audited against the previously established audit criteria, including audit standard, audit method, audit interval, audit form, and feedback system. Each profile prescription entry is evaluated and analyzed against the audit criteria for completeness, rational drug therapy and research protocols.

A second audit of fifteen randomly selected outpatient profiles was performed. Several observations were made, including: prescription entries in the profile are being completed appropriately in 90% of the instances; no significant drug-drug interactions were dispensed; no drugs were dispensed in violation of any known allergies or sensitivities; all prescriptions written and filled were within the boundaries of rational drug therapy; 93% of drugs written on research protocols were recorded in the appropriate log records; 69% of the prescriptions were dispensed with an indication if a consultation was performed by the pharmacist; problem numbers were indicated on 89% of prescriptions written.

The results of each audit are reviewed with the Clinical Research Pharmacy Service staff to discuss methods of improvement in the utilization of the outpatient drug profile to promote optimal patient care.

This study will continue with additional audits to be performed periodically. The results of subsequent audits will be compared against previous ones to determine if improvement has occurred.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01-CM 06961-01 COB

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Establishment of a Pharmacy Clinical Clerkship in Oncology

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Clarence L. Fortner, Director, Clinical Research Pharm. Service, CL-NCI
William R. Grove, Clinical Pharmacist, CL-NCI

OTHER: Robert Kerr, Pharm. D., Director, Clinical Pharmacy, University of MD
John Bender, Clinical Pharmacist, CL-NCI

COOPERATING UNITS (if any)

University of Maryland, School of Pharmacy

LAB/BRANCH
Clinical Oncology Branch

SECTION
Clinical Research Pharmacy Service

INSTITUTE AND LOCATION
NCI, NIH, Baltimore, Maryland 21201

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1	1	0

CHECK APPROPRIATE BOX(ES)

☑ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline ke

The BCRC pharmacy has joined with the University of Maryland School of Pharmacy in establishing a clinical clerkship in oncology for students obtaining their Doctor of Pharmacy Degree. This clerkship is an elective four week rotation.

The objective is to provide a clinical experience in oncology. The students are to understand the criteria for oncology research/treatment protocols, i.e. patient selection, study design and evaluation of study. They will gain experience in cancer therapy, toxicities and the management of non-neoplastic concurrent medical/social problems.

The pharmacy student should gain experience in both the inpatient and ambulabory areas.

PHS-6040
(Rev. 10-76)

The Clinical Research Pharmacy Service has joined with the University of Maryland School of Pharmacy in establishing a clinical clerkship in oncology for students obtaining their Doctor of Pharmacy Degree. This clerkship is an elective 4 week rotation.

The objectives are to provide a clinical experience in oncology. The students are to understand the criteria for oncology research/treatment protocols i.e., patient selection, study design and evaluation of study. They will gain experience in cancer therapy, toxicities and the management of non-neoplastic concurrent medical/social problems. They will learn the effective and reliable reference sources for the most current information on cancer chemotherapy.

The pharmacy student will gain experience in both the inpatient and ambulatory areas.

These students will be directed through the Clinical Research Pharmacy Service with support from the Medical Oncology Branch.

This is a new program which will continue for further evaluation.

LABORATORY OF CLINICAL BIOCHEMISTRY
July 1, 1976 to September 30, 1977

During the past year, the first year for the Laboratory of Clinical Biochemistry, efforts have been made to continue productive and exciting programs that were ongoing in the precursor Biochemistry Section; and new projects have been commenced which promise significant contributions in the areas related to the problem of cancer treatment. The Laboratory is concerned with the study of cellular control mechanisms, cellular organelle ultrastructure and function, drug disposition and metabolism, and mechanism of action.

In our studies on the biochemical pharmacology of antineoplastic agents and other drugs, we have continued a program in the study of carbonyl reductases. We have seen, that the reduction of the carbonyl group on xenobiotic substances occurs intracellularly and usually produces an active metabolic product which because of its different physical-chemical characteristics may produce prolonged or altered pharmacologic activity from the parent substance. In a study of xenobiotic substances, we found that carbonyl reductases occur almost exclusively in the cytoplasm of cells. Aromatic aldehydes such as benzyaldehyde, aromatic ketones such as acetophenone, aliphatic ketones such as oxisuran and naloxone, methyl ketones such daunorubicin and warfarin, tetranones such as bunolol, and unsaturated ketones are substrates of proven cytoplasmic activities that generate alcohol products. Of the few reductases that have been purified and studied, all of the enzymes have broad substrate specificities. The enzymes reduce xenobiotic carbonyls as well as naturally occurring substrates. These carbonyl reductases are widely distributed among tissues. This wide tissue distribution reflects the large reservoir of catalytic activity that is available. All the carbonyl reductases preferentially utilize NADPH as cofactor. The molecular weight range as well as their other physical-chemical properties suggest that the cytoplasmic carbonyl reductases are a family of enzymes. Certainly the similarities of the aldo-keto reductase from mammalian tissues are unlikely to be a matter of chance. From the collected studies, it appears that there is a system of related cytoplasmic aldo-keto reductases similar in several characteristics. These enzymes may be related genetically and through evolution, but these points remain for study. We have extended those studies further to investigate the exact differences of cytoplasmic carbonyl reductases and compared their heterogeneity in human, rabbit, rat, and mouse liver cytoplasmic fractions. Our experiments indicate that the enzymes are heterogenous and multiple forms of activities exist.

We have advanced our understanding of the microsomal reductive glycosidase, the microsomal metabolism of anthracyclines and other cancer chemotherapeutic drugs; and have been able to solubilize and isolate this important enzyme from the rat liver microsomes. We are currently determining the mechanism of action of this interesting and unique enzyme reaction. We have extended our studies of drug-drug interaction through work which bears clinical importance. The interaction of phenobarbital induction and anthracycline therapy show adriamycin therapy to have a decreased efficacy primarily through increased toxicity. Both metabolic and pharmacokinetic patterns for the adriamycin are changed by phenobarbital pretreatment. These findings suggest that drugs that induce the microsomal enzyme should not be used with adriamycin.

Another aspect of the microsomal studies has been the observation and investigation of the effect of anthracycline antibiotics on microsomal metabolism. We have demonstrated that the anthracyclines affect microsomes through an influence on microsomal electron transport. We have found that anthracyclines augment electron transfer from NADPH to oxygen to form superoxide. During this process free radical formation occurs and the anthracyclines exist in an intermediate stage as free radical forms. This observation contributes a new insight to understanding the mechanism of action of the anthracyclines. As free radical intermediates the anthracycline drugs may be potent reactants with intracellular components. We are continuing these studies and amplifying them with the intent to dissect this reaction and to extend its understanding and its utility into clinical studies.

In our continuing collaborative clinical studies with the Clinical Oncology Branch, BCRC we have investigated the human pharmacokinetics of adriamycin and daunorubicin by a newly developed radioimmunoassay and have compared the pharmacokinetics determined by this technique with the pharmacokinetics determined by the more classical fluorescence techinques and analyzed these data. We are continuing our interaction with the clinical program through collaborative clinical pharmacology studies of anthracyclines, the Cis-platinum compounds, and other newly developed anticancer agents. With respect to the potential clinical utility of hyperthermia, we have investigated this potential adjuvant therapy to understanding the cellular pharmacology of daunorubicin in L1210 leukemia cells. In our studies we show that although hyperthermia increases the uptake of daunorubicin over a narrow temperature range, it is additive in its effect with the daunorubicin toxicity to the L1210 cells. It may be possible by careful manipulation of temperature to improve antitumor activity. Our continuing studies on the interaction of the anthracyclines with nucleic acids have shown that the nucleic acids induce a DNA-strand breakage which can be determined by the Rydberg technique. These data also have shown that daunorubicin, adriamycin, actinomycin D, and bleomycin as well as procarbazine break DNA strands.

We have attempted to develop the other model systems to understand drug toxicity. In these studies, we have utilized new born chick and lepidoperan larva. In other studies on the leukemia and animal model systems, we determined the transplacental passage of the WEHI3 tumor viruses. These "C" type viruses presumably cause the formation of lymphomas in mothers and offspring after latency of 6 - 10 months. A preliminary study with chemotherapeutic effects of Levamisol showed no effect of this drug on the latency period in tumor development of WEHI3 tumor. In a collaborative with the Clinical Oncology Branch, we have carried out an investigation of the application of cationic anesthetics with respect to filtration leukopheresis and the use of tetracaine and lidocaine to reverse adherence of normal human granulocytes to nylon fibers. These studies suggest that this method could possibly be used in the application to filtration leukopheresis.

We have continued our collaborative efforts with other Federal and academic institutions. Some of the basic collaborative studies are as follows: mechanism of action of anthracycline antibiotic analogs, University of Southern California; development of radioimmunoassays, Brandeis University; comparative structure-activity relationships of cytotoxic antibiotics, Farmitalia and the Upjohn Company; etc. Examples of collaborative clinical studies are the clinical pharmacology of cancer chemotherapeutic studies, Clinical Oncology Branch, Radiatoin Therapy Branch, NCI: biochemical activities of human leukemia leukocytes, Hematology Division, Johns Hopkins Hospital, and Pathology Departments U.S. Public Health Service Hospital and Johns Hopkins Hospital, etc. Through these interactions we have developed rich resources for our research needs.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 06012-07 LCB

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Biochemical Pharmacology of Antineoplastic and Other Agents

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	N. R. Bachur	Chief, Lab. Clin. Bioch.	LCB, NCI
OTHER:	R. L. Felsted	Research Chemist	LCB, NCI
	M. J. Egorin	Clinical Associate	LCB, NCI
	H. Kubo	Visiting Fellow	LCB, NCI
	N. Ahmed	Visiting Fellow	LCB, NCI
	M. Kaber	Lab. Tech.	LCB, NCI
	M. Gee	Chemist	LCB, NCI
	S. Gordon	Chemist	LCB, NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Clinical Biochemistry

SECTION

INSTITUTE AND LOCATION
NCI, NIH, Baltimore, Maryland 21211

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
5.1	2.2	2.9

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The liver keto reductases which metabolize adriamycin and daunorubicin are heterogenous. Significant species differences in these enzymes are shown. However, the similarity of the subcellular distribution, broad substrate specificity, cofactor dependence, and physical chemical characteristics of the aldo-keto reductases suggest these to be a class of enzymes. Microsomes contain the enzymatic machinery to catalyze anthracycline dependent electron transport. In this process the anthracyclines are reversibly converted to free radicals.

Project Description:
Cytoplasmic aldo-keto reductases metabolize the anticancer chemotherapeutic antibiotics adriamycin and daunorubicin to their corresponding glycol or alcohol glycosides. A comparative study determined the heterogeneity of these reductases in human, rabbit, rat, and mouse liver cytoplasmic fractions. Evidence is provided for the existence of more than one form of adriamycin and daunorubicin reductases in rabbit and human. Different forms of adriamycin and daunorubicin reductases are resolved by ion exchange chromatography on DEAE cellulose columns. Isoelectric focusing resolves a pH 6.0 enzymatic activity from the pH 8.5 activity, and resolves multiple forms for each activity. This study shows that the rabbit and human are unique in sharing a prominent pH 6.0 enzymatic activity.

Other xenobiotic substances such as benzaldehyde, pNO_2 benzaldehyde, warfarin, bunolol, cyclohexanone, oxisuran, naloxone, naltrexone, and metyrapone are also preferentially reduced to alcohols by cytoplasmic enzymes in mammals. These enzymes are widely distributed in the tissues, have broad substrate specificities, have similar physical-chemical characteristics, and require reduced nicotinamide adenine dinucleotide as cofactor the the reductions. We propose that these reductases define a system of detoxification for aldehyde and ketone groups.

Adriamycin, daunorubicin, and other anthracycline antibiotics stimulate the microsome catalyzed consumption of both oxygen and NADPH. Rat liver microsomes and heart sarcosomes catalyze this process and exhibit augmentation of the electron flow by anthracycline antibiotics. This process is selectively dependent on NADPH (Km 4.2×10^{-4}M) and has an optimum pH of 8.0 - 8.5. Alpha tocopherol and parahydroxymercuriphenylsulfonic acid inhibit both anthracycline antibiotic stimulated and endogenous oxygen consumption. The Km's for anthracycline antibiotics approximate 10^{-4}M. ESR spectra show the formation of a free radical during the reaction which is absolutely dependent on the microsomes, NADPH, and anthracycline antibiotics. The anthraquinone nucleus of the anthracycline antibiotics is reversibly converted to a free radical semi-quinone which serves to shuttle electrons to oxygen. This enhanced free radical formation may function as the toxic and/or active principle of anthracycline chemotherapy.

The elucidation of the biotransformations of antineoplastic agents may be useful in understanding the pharmacodynamics and toxicity of these agents. Additionally, understanding the metabolic machinery can be useful in manipulating the affects of the drugs.

Publications:
Bachur, N.R. and Felsted, R.L.: Oxisuran reduction by rabbit tissue preparations. Drug Metab. Disp. 4: 239-243, 1976.
Bachur, N.R. and Gee, M.: Microsomal reductive glycosidase. J. Pharmacol. Exp. Therap. 197: 681-686, 1976.
Bachur, N.R.: Cytoplasmic aldo-keto reductases: A class of drug metabolizing enzymes. Science 193: 595-597, 1976.
Bachur, N.R.: Biochemical Pharmacology of the anthracycline antibiotics. ACS Symposium Series 300: 58-70, 1976.
Bachur, N.R.: Daunorubicin biotransformation #C 10337. In Verbiscar, A.J. (Ed.): Biotransformation Reviews. Sierra Madre, Anver Bioscience Design, 1976.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 06014-07 LCB

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Cellular Control Mechanisms Affecting Cell Growth and Differentiation

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	N. R. Bachur	Chief, Lab Clin Bioch.	LCB, NCI
OTHER:	M. J. Egorin	Clinical Associate	LCB, NCI
	R. L. Felsted	Research Chemist	LCB, NCI
	F. T. Sanel	Research Biologist	LCB, NCI
	R. N. Odders	Clinical Associate	LCB, NCI

COOPERATING UNITS (if any)
None

LAB/BRANCH
Laboratory of Clinical Biochemistry

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INSTITUTE AND LOCATION
NCI, NIH, Baltimore, Maryland 21211

TOTAL MANYEARS: 2.2	PROFESSIONAL: 2.2	OTHER: 0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The isolation of substances from plant and animal sources which effect the growth and differentiation of normal and malignant human cells is paramount in the study of the processes which control ceullular activities. By studying these materials as biochemical tools, we may be able to understand the biochemical mechanisms controlling cell proliferation and differentiation. Also, by investigating points where control of metabolic pathways are possible, we may find agents to control cellular growth, differentiation, and proliferation. We have established the chemical and biochemical characteristics of the mitogenic and agglutinating components of phytohemagglutin (PHA). The isolectins of PHA show a specificity to different blood cell membranes. The L component has mitogenic activity and agglutinates leukocytes. The E component has less mitogenic activity and agglutinates erythrocytes. The L_4 tetramer reversibly inhibits the growth of murine leukemia L1210. Rat liver stimulator substance has been partially characterized and studied with respect to the diurnal rhythm associated with normal rat liver DNA synthesis.

PHS-6040
(Rev. 10-76)

Project Description:

Phytohemagglutinin isolectins L4 and E4 stimulate and transform normal human and murine lymphocytes as well as those in chronic lymphocytic leukemia. In contrast, these proteins inhibit growth and proliferation of cultured L1210 murine leukemia cells. L1210 cells incubated with L4 or E4 show dose dependent inhibition of ^{3}H thymidine and ^{14}C uridine incorporation. L4 is 30-50 times more potent an inhibitor than is E4. Inhibition begins 2-3 hours after exposure of L1210 cells to L4 and persists for as long as cells are exposed to this isolectin. Total DNA in L4 treated cultures is correspondingly decreased. Protein synthesis is less affected, while glucose utilization remains unchanged. The binding of L4 and E 4 to L1210 cells and human lymphocytes is similar and is reversible by porcine thyroglobulin. Porcine thyroglobulin also reverses L4 induced inhibition of nucleotide incorporation. Cell aggregation is the only morphologic consequence of isolectin treatment observed by light or electron microscopy. No evidence of cell death as estimated by ^{51}Cr release or trypan blue uptake has been noted. Our data indicate that L4 and E4 have cytostatic properties and demonstrate that the reversible binding of a macromolecule to the surface of a malignant cell can modulate synthetic pathways and the rate of proliferation.

We have described and partially characterized an extract from weanling, or regenerating rat liver (SS) which stimulates incorporation of ^{3}H thymidine into liver DNA when injected i.p. into a one-third hepatectomized rat. It is heat stable, acid labile, is not dialyzable, and has a molecular weight of about 10,000. In further studies with a controlled light schedule, both SS activity and endogenous DNA synthesis of the donor weanling rats display a distinct diurnal rhythm with SS demonstrable during the peak of DNA synthesis (3-9am) and undetectable during the nadir (6-9 pm). SS does not appear to have a controlling role in normal DNA synthesis although it clearly stimulates regenerative growth. SS has been characterized to: 1) be organ specific with no effect on DNA synthesis of rat kidney, spleen or bone marrow; 2) be species non-specific since rat SS stimulates DNA synthesis in liver of normal CF$_1$ mice; 3) increase only DNA and not protein or RNA synthesis; 4) have no effect on DNA, RNA or protein synthesis in liver slices or liver parenchymal cell suspensions; 5) increase DNA synthesis in primary, non-proliferating, adult, rat liver parenchymal cell cultures after a 12 hour lag period.

We will continue our studies on the control mechanisms which affect cellular growth and differentiation, and attempt to elucidate control principles affecting all levels of cellular machinery. Specifically, we hope to purify hepatic stimulator substance and to describe its physiological action in the normal animal. We hope to elucidate the mechanism of action of the isomitogens on cell membranes. Thirdly, we will continue our search for materials characteristic of altered metabolic pathways, which may be used to quantify malignant cells *in vivo*.

Publications:

Felsted, R.L., Leavitt, R.D., and Bachur, N.R.: Precipitation reactions of red kidney bean (*Phaseolus vulgaris*) phytohemagglutin isolectins. *Comp. Biochem. Physiol.* 55B: 417-421, 1976.

Egorin, M.J., Felsted, R.L., and Bachur, N.R.: Phytohemagglutinin isolectin stimulation of glucose utilization by lymphocytes. *Life Sciences* 20: 351-358, 1977.

Leavitt, R.D., Felsted, R.L., and Bachur, N.R.: Biological and biochemical properties of *Phaseolus vulgaris* isolectins. *J. Biol. Chem.* 252: 2961-2966, 1977.

Felsted, R.L., Egorin, M.J., Leavitt, R.D., and Bachur, N.R.: Recombinations of subunits of *Phaseolus vulgaris* isolectins. *J. Biol. Chem.* 252: 2967-2971, 1977.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 06016-07 LCB

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Pharmacodynamics of Cancer Chemotherapeutic Agents

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	N. R. Bachur	Chief, Lab Clin Bioch.	LCB, NCI
OTHER	C. A. Padgett	Clinical Associate	LCB, NCI
	M. E. Klein	Clinical Associate	LCB, NCI
	S. Gordon	Chemist	LCB, NCI
	M. F. Goodman	Univ. Southern Calif.	

COOPERATING UNITS (if any)

Department of Biology, University of Southern California

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Laboratory of Clinical Biochemistry

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NCI, NIH, Baltimore, Maryland 21211

TOTAL MANYEARS: 1.8	PROFESSIONAL: 1.7	OTHER: 0.1

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Studies on drug effected DNA strand breakage in cultured L1210 cells show that the hydroxyl apatite chromatography method can be utilized for quantitation of this action. The anthracycline antibiotics, actinomycin D, and bleomycin showed extensive DNA strand breakage as did hyperthermia. Hyperthermia gives additive effect to anthracycline therapy against L1210 cells in culture. Our studies on the mechanism of action of adriamycin and daunorubicin against T4 DNA polymerase show both uncompetitive and competitive inhibition at low and high drug to DNA ratios. This can be related to intercalative and ionic binding of adriamycin to DNA.

PHS-6040
(Rev. 10-76)

Project Description:

The time course of drug-induced DNA strand breakage was examined by measurement of the rate of DNA unwinding in alkalai. L1210 cells grown in culture were assessed for DNA single strand as a measure of unwinding (F60). 10^{-6} molar daunorubicin caused a fall in F60 with time (77% control values at 6 hours). 10^{-8} and 10^{-7} molar daunorubicin resulted in an initial rise in F60 that returned to control by six hours. Actinomycin D gave similar curves, but adriamycin caused only slight damage over the six hour incubation. 10^{-8}, 10^{-7}, and 10^{-6} molar procarbazine decreased F60 to 82% by five hours. Bleomycin (15 micrograms/ml) and 1 mM potassium cyanide both decreased F60 to 84% at four hours, but heating cells to 55° for ten minutes caused extensive and progressive DNA damage over the four hour test period.

Data on the interaction of hyperthermia with daunorubicin were obtained from L1210 leukemia cells. Cells were incubated with daunorubicin at pH 7.4 for one hour at temperatures of 37, 38.5, 39, and 40°C. Fluorometric measurement of daunorubicin uptake at 10^{-4} M showed increases over 37° values for 38.5, 39, and 40°C. Maximal inhibition of tritiated thymidine incorporation occurred with the 10^{-4} M concentration and was temperature independent. Values approaching maximal inhibition were also seen with 1 and 2 x 10^{-5} M concentrations incubated at 39 and 40°C. These results indicate that the inhibitory effect of low dose daunorubicin approaches that of higher doses when temperatures are increased to 39 and 40°C, and the cellular uptake of daunorubicin increases with increased temperature. It may be that increased antineoplastic activity can be obtained from the anthracyclines by manipulating a patient's temperature, and better antitumor activity can be obtained prior to the onset of irreversible anthracycline induced cardiotoxicity.

We examined the effect of adriamycin on kinetics of DNA synthesis catalyzed by a DNA polymerase purified from bacteriophage T4-infected Escherichia coli. Two distinct modes of enzyme inhibition occur: uncompetitive and competitive at "low" and "high" drug:DNA nucleotide molar ratios, respectively. Competitive inhibition is not observed unless an unblocked amino group is present on the sugar (daunosamine) moiety. A model is proposed to relate the enzyme inhibition kinetics to intercalative and ionic binding of adriamycin to DNA.

To investigate the biochemical and physiological effects of antineoplastic drugs and their mechanism of action. To correlate the effects and activities of the drugs with their chemical structures.

Understanding the structure activity relationships of drugs is useful in planning new improved agents. Further explanations through study of the pharmacodynamics can help clarify problems in chemotherapy.

Publications:

Bachur, N.R., Steele, M., Meriwether, W.D., and Hildebrand, R.C.: Cellular pharmacodynamics of several anthracycline antibiotics. J. Med. Chem. 19: 651-654, 1976.

Reich, S.D. and Bachur, N.R.: Alterations in adriamycin efficacy by phenobarbital. Cancer Res. 36: 3803-3806, 1976.

Goodman, M.F., Lee, G.M., and Bachur, N.R.: Adriamycin interactions with T4 DNA polymerase: Two modes of template mediated inhibition. J. Biol. Chem. 252: 2670–2674, 1977.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 06241-07 LCB

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Clinical Pharmacology of Antineoplastic and Other Drugs

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	N. R. Bachur	Chief, Lab. Clin. Bioch.	LCB, NCI
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	M. E. Klein	Clinical Associate	LCB, NCI
	P. H. Wiernik	Chief, Clin. Oncol Br.	CL, NCI
	P. Chang	Clinical Associate	CL, NCI
	H. Van Vunakis	Brandeis University	

COOPERATING UNITS (if any)
Clinical Oncology Branch, Baltimore Cancer Research Center, NCI
Biochemistry Department,Brandeis University

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Laboratory of Clinical Biochemistry

SECTION

INSTITUTE AND LOCATION
NCI, NIH, Baltimore, Maryland 21211

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.4	0.4	

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Our sustained investigation of the clinical pharmacology of antineoplastic agents continues to aid in the understanding of clinical problems through fundamental approaches. Adriamycin antitumor efficacy is reduced by pretreatment of mice with the microsomal inducer phenobarbital. The phenobarbital pretreatment alters the disposition and metabolism of adriamycin. A radioimmunoassay for adriamycin and daunorubicin showed that the pharmacokinetics of these agents can be determined. However, the values differ from those obtained by other methods.

PHS-6040
(Rev. 10-76)

Project Description:
Adriamycin dosage should be reduced in patients with impaired liver function, since adriamycin disposition is influenced by liver metabolism and biliary excretion. It follows that drugs that increase the metabolism or excretory capacity of the liver may decrease adriamycin concentrations to suboptimal values. Adriamycin metabolism was therefore studied in mice pretreated with phenobarbital (75 mg/kg i.v.) by injection. After an i.v. dose of adriamycin (30 mg/kg i.v.), plasma fluorescence due to drug and metabolites was less and disappeared at a greater rate in phenobarbital-pretreated mice than control animals. When extracted, the livers from the phenobarbital-pretreated group yielded a greater concentration of aglycones. Experiments with liver microsomes confirmed that aglycone production occurred at a more rapid initial rate in phenobarbital-induced livers. No increase in aldo-keto reductase (daunorubicin reductase) activity occurred. Phenobarbital-pretreated mice, inoculated i.p. with 1 million L1210 cells and then treated with adriamycin (6 mg/kg i.v.), had significantly lower survival than did control (p 0.01). These findings show that phenobarbital affects the disposition of adriamycin by microsomal enzyme induction and suggest that drugs that induce microsomal enzymes should not be used concurrently with adriamycin if optimal drug efficacy is desired.

Our cooperative study of acute nonlymphocytic leukemia remission revealed that no relationship was discerned between concentrations of kinase, deaminase, kinase to deaminase ratio, or daunorubicin reductase and attainment of complete remission, duration of complete remission, or survival from therapy in any of the three treatment groups. This study fails to confirm any usefulness of these enzyme assays in predicting attainment of complete remission with either cytosine arabinoside or daunorubicin alone, or cytosine arabinoside and daunorubicin together.

In 38 adriamycin treated patients and 4 daunorubicin treated patients, radioimmunoassay readily and reproducibly detects and estimates these drugs and immunologically similar metabolites in patients' plasma and urine to at least 120 hours after dosing without interference by concurrent medication. The plasma drug decay follows first-order kinetics in a triphasic pattern. Radioimmunoassay and fluorescence assay show similar decay up to four hours but diverge at that point with the fluorescence assay yielding higher values. Pharmacokinetic differences are amplified in patients with liver dysfunction and may be caused by fluorescent drug metabolites not sensitive to radioimmunoassay or nonspecific fluorescent materials. The radioimmunoassay offers the capability to measure adriamycin and daunorubicin in clinical settings in which fluorescence assay is not available.

Publications:
Chang, P., Riggs, C.E., Sheerer, M.T., Wiernik, P.H., and Bachur, N.R.: Combination chemotherapy with adriamycin and streptozotocin. II. Clinical-pharmacological correlation of augmented adriamycin toxicity caused by streptozotocin. Clin. Pharmacol. Therap. 20: 611-616, 1976.

Bachur, N.R., Riggs, C.E., Green, M.R., Langone, J.J., VanVunakis, H., and Levin, L.: Human plasma adriamycin and daunorubicin by fluorescence and radioimmunoassay. Clin. Pharmacol. Therap. 21: 70-77, 1977.

Benjamin, R.S., Riggs, C.E., and Bachur, N.R.: Plasma pharmacokinetics of adriamycin and its metabolites in humans with normal hepatic and renal function. Cancer Res. 37: 1416-1420, 1977.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 06250-04 LCB

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

TEM, SEM, and Culture of Human Leukemic Cells and of Normal Donor Granulocytes for Transfusion Therapy

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	F. T. Sanel	Research Biologist	LCB, NCI
OTHER:	P. H. Wiernik	Chief, Clin. Oncol.	CL, NCI
	J. Aisner	Clinical Associate	CL, NCI
	C. A. Schiffer	Clinical Associate	CL, NCI
	M. J. Egorin	Clinical Associate	LCB, NCI
	R. L. Felsted	Research Chemist	LCB, NCI
	N. R. Bachur	Chief, Lab Clin. Bioch.	LCB, NCI

COOPERATING UNITS (if any)

Clinical Oncology Branch, Baltimore Cancer Research Center, NCI

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Laboratory of Clinical Biochemistry

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INSTITUTE AND LOCATION
NCI, NIH, Baltimore, Maryland 21211

TOTAL MANYEARS: 3/8	PROFESSIONAL: 1/8	OTHER: 1/2

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Elution of normal granulocytes from nylon fibers, using cationic anesthetics, indicate that tetracaine-eluted cells were comparable to non-filtered controls in viability, micromorphology, and phagocytic competence. Detachment from the fibers by these agents appears to occur by a mechanism not damaging to plasma membranes and cell constituents.

PHS-6040
(Rev. 10-76)

Project Description:
Continuing studies are directed toward the characterization of leukemias of uncertain identity by ultrastructure, cytochemistry and function of peripheral blood and bone marrow cells. Hairy cell leukemia should be definitively identified in suspected cases since the prognosis for untreated patients with this disease is considerably better than for those treated chemotherapeutically.

The potential application of cationic anesthetics to filtration leukopheresis was investigated with tetracaine and lidocaine to reverse adherence of normal human granulocytes to nylon fibers in a miniaturized system. Harvested cells were examined for viability, ultrastructure phagocytic competence and histochemically for glycogen and peroxidase localization. Comparison with normal nonfiltered control and with cells harvested by conventional procedures that utilize calcium chelating agents indicated that tetracaine eluted cells were similar to normal controls; electron micrographs suggest that detachment from the nylon fibers may be accomplished by a mechanism not damaging to plasma membranes or cell constituents.

In order to determine the role of several components of complement in granulocyte adhesion to nylon fibers conventionally used in continuous flow filtration, leukocytes from two mouse strains genetically deficient in C 5 were tested in a miniturized model system. Granulocyte adhesion occurred but the significance of differences between controls and deficient species remain to be evaluated.

We will continue our investigation of methods to harvest donor cells and restore the maximal viability and efficiency of granulocytes collected for transfusion therapy.

Publications:
Sanel, F.T., Aisner, J., Schiffer, C.A.: Filtration leukopheresis. Blood 47: 877-878, 1976.
Schiffer, C.A., Sanel, F.T., Young, V.M., Aisner, J.: Reversal of granulocyte adherence to nylon fiters by local anesthetics: Possible application to filtration leukopheresis. Blood, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 06272-06 LCB

PERIOD COVERED

July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Studies of Leukemia in Animal Model Systems

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	F. T. Sanel	Research Biologist	LCB, NCI
OTHER:	P. H. Wiernik	Chief, Clin. Oncol. Br.	CL, NCI
	A. Pollak	IPA	LCB, NCI
	R. Clawson	Biologist	LCB, NCI
	M. J. Egorin	Clinical Associate	LCB, NCI
	N. R. Bachur	Chief, Lab Clin. Bioch.	LCB, NCI

COOPERATING UNITS (if any)

Clinical Oncology Branch, Baltimore Cancer Research Center, NCI

LAB/BRANCH

Laboratory of Clinical Biochemistry

SECTION

INSTITUTE AND LOCATION

NCI, NIH, Baltimore, Maryland 21211

TOTAL MANYEARS: 1/2	PROFESSIONAL: 1/8	OTHER: 3/8

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Transplacental passage of WEHI-3 tumor viruses induce lymphomas in offspring suggesting that immunologic responses to them have not been elicited; adult BALB/c mice cannot reject these tumor-inducing viruses; these viruses are in some instances sequestered and/or produced within the bone matrix; demyelinization of spinal cord axons and the presence of virus in the spinal cord may be related phenomena in animals that develop the hind leg paralysis.

Preliminary results of administration of adriamycin to normal newborn chicks suggest non-specific toxicity with occasional pericardial, myocardial and heptaic lesions. In the invertebrate model, normal development of the larva is seriously impaired and lesions in the thoracic dorsal vessel (analogue to the vertebrate heart) are similar to those reported in adriamycin induced cardiac toxicity in humans. The value of using these models remains to be evaluated.

PHS-6040
(Rev. 10-76)

Project Description:

Scanning electron microscopy (SEM) of tissue cultured WEHI-3 tumor cells confirmed their identity as monocyte-macrophage type cells and transmission (TEM) demonstrated their production of "C" type particles, previously tested in tumor extracts that demonstrated group specific antigens of the FMR group and demonstrated high titers of infectivity. Newborn BALB/c mice injected with NB tropic cell-free tumor filtrates in many instances developed hind leg paralysis and lumbar spinal cord samples revealed moderate demyelinization and extracellular C-type viruses. TEM of decalcified femurs of osteopetrotic animals revealed numerous C-type particles in lacunar areas where osteocytes were embedded in bone matrix. Transplacental transmission of viruses by injection of pregnant females with cell free filtrates (CFF) resulted in lymphomas in both mothers and offspring after latencies of 6 to 10 months. Injection of CFF into normal 10 month old BALB/c males induced lymphomas after a 6 to 8 month latency. In preliminary chemotherapeutic studies with Levamisole (Ortho), the drug was administered ip, in saline, 5 g per mouse at staged intervals before tumor grafting, simulataneous with tumor grafting, and after tumor grafting, and/or in multiple doses of 5 g each. Latency periods of tumor development did not significantly differ from tumor injected controls.

These studies have established that transplacental passage of WEHI-3 tumor viruses induce lymphomas in offspring suggesting that immunologic responses to them have not been elicited; that adult BALB/c mice cannot reject these tumor-inducing viruses, unlike adult AKR mice injected with Gross virus; that these viruses are in some instances sequestered and/or produced within the bone matrix, and that demyelinization of spinal cord axons and the presence of virus in the spinal cord may be related phenomena in animals that develop the hind leg paralysis.

We will continue to study the oncogenic expression of these viruses and will investigate *in vivo* and *in vitro* chemotherapeutic anti-viral and anti-neoplastic agents.

The side effects of adriamycin are under study in two non-mammalian animal models, the chick and a lepidopteran larva. Developmental stages in the insect are compared with normals and tissues in both models are examined histologically, ultrastructurally and by fluorescence microscopy, the last to determine drug uptake and its localization. Preliminary findings in the newborn chick indicate a non-specific toxicity and in many instances recovery from initial weight loss. Histologic evidence of pericardial and myocardial damage is observed in some animals but generally chicks do not respond consistently with given doses at specific ages. In the invertebrate model, normal development through pupal and final metamorphosis stages is seriously impaired, with consistent weight loss after a single dose of the drug. Multiple doses or a massive single dose induce changes in the dorsal vessel (analogue of the heart) and comparable to lesions reported in humans and mice with adriamycin and daunorubicin induced cardiac toxicity. These consist of disrupted myofibrils, alterations in the mitochondria and focal disruption of the normal architecture of the organ.

SUMMARY REPORT

LABORATORY OF MOLECULAR BIOLOGY

July 1, 1976 through September 30, 1977

One major area of concentration within the Laboratory of Molecular Biology has been the study of cellular regulation particularly as it effects those factors which control the stabilization of messenger RNA (mRNA) in normal and neoplastic tissues. In this connection, the significance of polyadenylic acid (poly (A)), a structure found at the 3' terminus of many mRNAs as well as viral RNAs, has been the subject of an intensive study in this Laboratory. Previously, we had shown that polyadenylic acid is a competitive inhibitor of many ribonucleases and more recently an attempt has been made to study the function of poly (A) at the termini of mRNAs within three specific tissues: bovine muscle, amphibian and avian livers. These tissues were chosen because preliminary studies have indicated that they contain a wide assortment of RNases, all of which are capable of degrading mRNA. In each case, the enzymes in the tissues were isolated and characterised, and a particular mRNA from each of the tissues has also been isolated. The reasoning being that if the mRNA were stable in the presence of enzymes to which they would theoretically be exposed, then a reasonable model could be constructed to examine the mechanisms of polyadenylic acid action. Thus, it was found that if polyadenylic acid were removed from mRNA and the messenger exposed to ribonucleases from the same tissue, the messenger would be rapidly degraded. On the other hand, if the mRNA containing a full complement of poly A were exposed to the same enzyme, no degradation took place. An important factor in the stabilization of mRNA then would appear to be the presence of polyadenylic acid at its 3' terminus.

One additional aspect of all this is the fact that the polyamine spermidine can reverse the inhibition by poly (A) of RNase activity. This is important, in that it provides a means whereby a messenger RNA can be degraded; a degradation which, in fact, takes place in the cell after a suitable period of protein synthesis. Thus, after production of new proteins there occurs a build up of spermidine such that eventually the RNase bound to poly (A) is liberated and proceeds to digest the mRNA. Preliminary studies are presently underway examining this mechanism in neoplastic tissues, as well.

Another aspect of work on ribonucleases is the apparent elevation of activity of a serum ribonuclease in response to kidney diseases. Although it was thought at one time, that elevations of this enzyme was a direct result of neoplasia, it has been shown that at least with respect to multiple myeloma and various leukemias, it is the damage to the kidneys which produces the increments in serum RNase activity.

The Section of Immunology and Cell Biology has continued to develop information concerning the characteristics of immunologically induced in vitro lymphocyte responsiveness. Advantage has been taken of the ability to measure alteration of laser light transmission by single viable unstained cells and to characterize and define cellular changes during the blastogenesis response. It was ascertained that laser cytometry was capable of characterizing changes within the lymphocyte population with great rapidity. Comparison of laser analysis of human lymphocyte responsiveness to the standard ^{3}H thymidine assay as well as morphologic analysis of the lymphocyte population indicated a marked discordance between actual lymphocyte proliferation and that proliferation suggested by thymidine incorporation. Active cell proliferation continued in the presence of significantly diminished thymidine incorporation indicating that exogenous thymidine was not being utilized by the cells as had previously been thought. Investigation of agents which can modulate immune responsiveness indicated that various polynucleotides had the ability to amplify _in vitro_ lymphocyte responsiveness to specific antigen and that the beta blockers, propranolol and dichloroisoproterenol also had this ability. The role of immunotherapy as part of a combined modality program in the treatment of B16 melanoma was carried out. Both chemotherapy as well as immunotherapy were used as adjuvants to surgery in this situation. The sequence of chemotherapy and immunotherapy as adjuvants to surgery proved important. Chemotherapy was significantly better when given before surgery, immunotherapy was more effective when delivered after surgery. The most effective therapeutic program was a single course of chemotherapy preceding surgery followed by immunotherapy. Studies relating to lymphocyte responsiveness to viruses indicated that the blastogenesis assay might be capable of predicting those patients with Hodgkin's disease who might be susceptible to the varicella zoster virus. When compared to normal donors, patients with Hodgkin's disease in whom radiotherapy was recently completed and those with active recurrent disease were found to have markedly impaired cell associated immunity to the varicella zoster antigen. Although newly diagnosed untreated patients with Hodgkin's disease did not differ significantly from normal persons as a group, two of six were found to be unresponsive to the varicella zoster antigen. Additional evidence concerning the association of elevated nucleolytic activity with the cancer and precancer state evolved. Analysis of serum alkaline ribonuclease activity of 500 cancer patients and 168 healthy donors was carried out. In the 11 malignancies studied, a significant difference in nucleolytic activity ($p<0.001$) between the cancer patients and the normal control group was noted. Of importance was the observation that abnormally elevated activity against one synthetic substrate did not necessarily indicate elevated activity against all.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 06242-07 LMB

PERIOD COVERED
July 1, 1976, to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Polyamines and Cancer; Functional Significance

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	T.P. Karpetsky	Staff Fellow	LMB, NCI
OTHER:	C.C. Levy	Chief	LMB, NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Molecular Biology

SECTION

INSTITUTE AND LOCATION
NCI, NIH, Baltimore, Maryland 21211

TOTAL MANYEARS: 0.7	PROFESSIONAL: 0.4	OTHER: 0.3

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The thermal inactivation of a Ctirobacter sp. ribonuclease (RNase) is subject to control by a number of factors. Low concentrations of naturally occurring polyamines such as spermidine and spermine, and certain analogs of these compounds, protect the enzyme from inactivation. Changes in ionic strength cause wide variations in the rate at which enzyme activity is lost. Additionally, depending on the type of ion added to the reaction mixture, the rate constant for enzyme inactivation may either increase or decrease as the ionic strength is raised. Thermodynamic parameters were determined under a variety of experimental conditions for the thermal inactivation of this RNase. It was found in all of these cases that the entropy of activation is large and negative, implying that a gross change in enzyme conformation is not taking place. The concentration and identity of ions present and the amount of polyamine available to interact with this RNase determines the rate of loss, by thermal inactivation, of enzyme activity in this in vitro system. These factors therefore constitute a system whereby substrate hydrolysis may be controlled with time.

PHS-6040
(Rev. 10-76)

Control of polynucleotide levels over time may be achieved by utilizing a ribonuclease (RNase) that undergoes inactivation at a rate dependent upon factors in the environment of the enzyme. The rate of thermal inactivation of an RNase from Citrobacter is controlled by a number of factors, but for a great variety of experimental conditions, may be described by pseudo first order kenetics. Alterations in either the type or concentration of ions present in the reaction mixture are reflected in different rates of loss of enzyme activity. These ionic effects are specific and conform to laws governing electrostatic interactions, as in the case of the buffers, Tris-HCI or potassium phosphate. Increasing concentrations of potassium phosphate buffer, however, induce a decrease in the rate of thermal inactivation, while elevations of Tris-HCI levels have an opposite effect. The buffering ions, therefore, would seem to be playing two distinct roles in the inactivation process. On the one hand, because of the specific effect, the identity of the buffering salt apparently determines the nature of the ions or residues participating in the rate-limiting step for inactivation. Moreover, increasing concentrations of these salts modify, by an identical electrostatic mechanism, the activity coefficients of the ions involved in the inactivation mechanism. Alterations in the rates of inactivation are also induced by concentrations of certain polyamines that do not influence the overall ionic strength. Studies on aliphatic and heterocyclic analogs of the polyamine spermidine indicated that considerable variation in the pK_a for the amino groups analogous to those of spermidine does not result in a loss of efficacy as long as the nitrogen corresponding to the secondary nitrogen of spermidine is not quanternized. There is a certain degree of freedom available in the lengths of the carbon chains between the nitrogens of the polyamine structure. Furthermore, these nitrogens may be substituted and the structure rigidified only if the bulk of the molecule is so placed to allow for free access, by the enzyme, to these heteroatoms. The rather sensitive response of the rate of inactivation of the enzyme to alterations in the type and concentration of ions present suggeststhat a relatively minor, but environmentally sensitive, change in overall conformation accompanies loss of enzyme activity. The ease with which the inactivated enzyme may be reactivated under certain conditions and the thermodynamic constants for the inactivation process, particularly the large, negative entropies of activation, support the view that a minor change in protein structure is taking place, in contrast with a major alterations, such as protein unfolding. The above data supports a novel interaction between polyamine and ribonucleases whereby the activity of the enzyme, and therefore, the level of substrate, may be regulated over time. The application of these findings to other ribonucleases to determine the generality of this effect is underway.

Publications:

Karpetsky, T.P. and Levy, C.C.: Thermal Inactivation of a Citrobacter Ribonuclease: Influence of Polyamines and Ionic Strength. Arch. Biochem. Biophys. 182 (pagination available in July), 1977.

Karpetsky, T.P., Hieter, P.A. and Levy, C.C.: Role for Polynucleotides as Inhibitors of Endonuclease Activity. In Proceedings of Second Conference on Modulation of Host Resistance in the Prevention or Treatment of Induced Neoplasms. Chirigos, M.A. (Ed.) New York, Raven Press, 1977, pp. 517-519.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 06247-07 LMB

PERIOD COVERED
July 1, 1976, to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Purification and Study of Nucleases with Differing Residue Specificities

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	M. Schmukler	Senior Investigator	LMB ,NCI
OTHER:	C.C. Levy	Chief	LMB, NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Molecular Biology

SECTION

INSTITUTE AND LOCATION
NCI, NIH, Baltimore, Maryland 21211

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.5	.75	.75

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project has three primary aims: first, and originally, to seek nucleases, which by virtue of their specificity, would be useful as tools in sequencing nucleic acids; second, to study the factors involved in regulation of nucleic acid metabolism; and third, to study alterations in nucleic acid metabolism in malignancy in order to determine whether any of these changes could serve as indicators for the presence or absence of disease.

Further studies have been conducted with plasma ribonuclease. Some modifications of the preparation procedures result in the production of three to four separate enzyme peaks. These different peaks are being studied to determine whether they are consistent and are different forms of the same enzyme. Blood from patients with malignant diseases is also being studied in this way to see if differences can be found in malignant and non-malignant plasma.

Publications:

Pendergrast, W.J.Jr., Drake, W.P., Schmukler, M. and Mardiney, M.D.Jr. Correlation of Abnormal Profiles of Nucleolytic Activity to Cancer, In Control of Neoplasia by Modulation of the Immune Systems. Ed. Chirigos, M.A., New York, Raven Press, 1977.

Neuwelt, E.A., Schmukler, M., Nizick, M.S., Jewett, P.B., and Levy, C.C. The Immunological Characterization of Several Human Ribonucleases Using Primary Binding Tests. Biochem. J., in press, 1977.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 06904-05 LMB

PERIOD COVERED
July 1, 1976, to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Anti-Tumor Activity in Nitrosoureas at the Subcellular Level

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	John Hilton, Ph.D.	Visiting Associate	LMB, NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Molecular Biology

SECTION

INSTITUTE AND LOCATION
NCI, NIH, Baltimore, Maryland 21211

TOTAL MANYEARS: 0.6	PROFESSIONAL: 0.4	OTHER: 0.2

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The half-lives of chloroethyl isocyanate and cyclohexyl isocyanate have been determined. Based on the half-lives it is possible to calculate the isocyanate concentration present during exposure of cells to BCNU and CCNU. Nitrosourea concentrations which are highly toxic to L1210 cells generate isocyanate levels which show little toxicity.

Project Description:

The role of the isocyanates released during the chemical breakdown of the chemotherapeutic nitrosoureas remains a speculative issue. Some observations reported in the literature have indicated that the nitrosourea-derived isocyanates may contribute to the overall chemotherapeutic action of the parent compounds. Others have related isocyanate generation to host toxicity. We have determined the half-lives of chloroethyl and cyclohexyl isocyanates (the isocyanates derived from BCNU and CCNU respectively) in tissue culture medium at 37 degrees. While the half-lives of chloroethyl isocyanate is extremely short, cyclohexyl isocyanate has a relatively long half-life. Calculation shows that if the initial nitrosourea concentration is 50 μM, the chloroethyl isocyanate and chclohexyl isocyanate concentrations are approximately constant at 0.2 μM and 2 μM respectively during the first hour of nitrosourea breakdown. A range of isocyanate concentrations have been maintained for 50 minutes in L1210 cultures and the cell viability determined by the soft agar cloning assay. Theeffect of the parent nitrosoureas (BCNU and CCNU) on L1210 viability was determined concurrently with the isocyanate studies. Our data indicate the isocyanates to be relatively non-toxic agents when presented at concentrations estimated to be generated by highly toxic levels of their parent nitrosoureas.

Publications:

Hilton, J., Bowie, D.L., Gutin, P.H., Zilo, D.M. and Walker, M.D. DNA Damage and Repair in L1210 Cells Exposed to CCNU. Cancer Research, in press, 1977).

Hilton, J. and Walker, M.D. DNA strand Scission and Its Repair Following Exposure of Cells to Inhibitors of Oxidative Phosphorylation. Biochem. Biophys. Res. Commun., 75: 909-914, 1977.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 06925-04 LMB

PERIOD COVERED

July 1, 1976, to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Studies on the Metabolism Function and Storage of Human Blood Platelets

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	M. Schmukler	Senior Investigator	LMB, NCI
OTHER:	C.C. Levy	Chief	LMB, NCI
	P.D. Zieve	Chief	Dept. of Medicine Baltimore City Hospitals

COOPERATING UNITS (if any)

Department of Medicine, Baltimore City Hospitals, Baltimore, MD 21224

LAB/BRANCH

Laboratory of Molecular Biology

SECTION

INSTITUTE AND LOCATION

NCI, NIH, Baltimore, Maryland 21211

TOTAL MANYEARS: 1.25	PROFESSIONAL: .75	OTHER: 0.5

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

These studies are designed to achieve several objectives: first, to obtain metabolic, physiologic, and pharmacologic information which would aid in the preservation and transfusion of stored platelets; second, to study the relation of various biochemical and pharmalogical factors on the function of platelets as measured by the release reaction; third, to elucidate the mechanism of the release reaction; and fourth, to gain general information about the mechanism of membrane-mediated cellular reactions.

PHS-6040
(Rev. 10-76)

The effect of L_4 and E_4 fractions of PHA on platelets has been studied with highly purified fractions. E_4 is consistently more potent than L_4 in its effect upon aggregation and release and binds more strongly to the platelet. Both show a release pattern similar to con A which was studied previously. Future studies are planned to identify and analyze the release receptors.

Publications:

Schmukler, M., Zieve, P.D., and Jewett, P.B.: Induction of the Platelet Release Reaction by Conconovalin A: Relation of Con A Binding to Release and Modification of Release by ATP, PGE, and Amantadine. Biochem. Parm. 25: 1819-1824, 1976.

Schiffer, C.A., Whitaker, C.L., Schmukler, M.,Aisner, J., and Hilbert,S.L. The Effect of Dimethyl Sulfoxide on *in vitro* Platelet Function. Thrombosis and Haemostasis 36: 221-229, 1976.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 06926-04 LMB

PERIOD COVERED

July 1, 1976, to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Role of Polyamines in Regulating Activity and Specificity of Various Nucleases

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	C.C. Levy	Chief	LMB, NCI
OTHER:	T.P. Karpetsky	Staff Fellow	LMB, NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Molecular Biology

SECTION

INSTITUTE AND LOCATION

NCI, NIH, Baltimore, Maryland 21211

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.7	0.4	0.3

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline k

Although several possible functions for the 3' terminal polyadenylic acid (poly A) portion of messenger RNA (mRNA) have been suggested, the experimental evidence at this time favors a stabilization role forthis homopolymeric segment. Thus, the amount of ribonuclease (RNase) mediated hydrolysis of the non-polyadenylated portion of mRNA is an inverse function of the length of the poly A segment. This novel finding is not restricted to a single enzyme. The activity of RNases from a number of sources (human spleen, human plasma, bovine pancreas, Enterobacter sp., and Citrobacter sp.) is inhibited by a 3' terminal segment of poly A. This inhibition is subject to a number of controls that regulate the amount of hydrolysis of the non-polyadenylated portion of the molecule. Small increases in ionic strength, for example, induce reversal of inhibition. Certain polyamines (spermidine and spermine) and analogs of these compounds were also effective in this respect. (The total ionic strength of the reaction mixture was not affected by the concentrations of the compounds added.) Polyamines also serve to protect the enzymes from thermal inactivation and to renature inactivated enzyme. Thus, the level of intact substrate is determined by a combination of factors: (1) length of the 3' terminal poly A portion, (2) ionic strength, and (3) polyamine concentration.

The results of many experiments performed in these laboratories and elsewhere, have indicated thatthe 3' terminal poly A segment of mRNA is responsible, in some fashion, for maintaining the integrity of the remainder of the mRNA molecule. Based on the finding that purine homopolymers, such as poly A, inhibit the activity of a number of ribonucleases, it seemed reasonable to assume that the mechanism by which poly A stabilizes mRNA is by inhibition of RNase mediated hydrolysis. This, in fact, was found to be the case for the interaction of model mRNAs and an RNase from human spleen; the longer the segment of3' terminal poly A, the greater the inhibition of RNase activity, with concomitant reduction in hydrolysis of the non-polyadenylated portion of the molecule. This would seem to be an excellent model for the mechanism by which tracts of poly A stabilize mRNA. It was the purpose of this investigation to extend these observations with the intent of answering two specific questions: (1) were these observations unique to the human spleen enzyme, or can they be generalized to a broad spectrom of RNases? (2) what factors influence or control the poly A induced inhibition of RNase activity? To probe these issues, a model system was constructed. Nine species of E. coli (^{3}H) 5S rRNA containing 3' terminal tracts of poly A ranging in size from 5 to 150 nucleotides in length were synthesized, using primer dependent polynucleotide phosphorylase. The poly A portion of these RNAs protect the remainder of the molecules from hydrolysis by RNases from various sources (human plasma, human liver, bovine pancrease, Citrobacter sp., and Enterobacter sp.). This homopolymeric purine segment is a very effective inhibitor, sequences as few as 28 adenylic acid residues induce greater than an 80% inhibition in the systems examined. Additionally, the poly A segments of these model substrates were capable of protecting from hydrolysis other substrate molecules (such as poly C or 5S RNA) lacking 3' terminal poly A tracts. Small increases in ionic strength cause reversal of inhibition with concomitant substrate hydrolysis, indicating the strong electrostatic nature of the interaction between the poly A tract and the RNase. An effect quite distinct from this is the polyamine mediated reversal of poly A induced inhibition of RNase activity. This induction of hydrolysis of substrate occurs if very low concentrations (5×10^{-5}M for A59) of certain polyamines, having no influence on total ionic strength, are utilized. The magnitude of this effect is related to the structure of the polyamine. Although, for example, spermidine, spermine and 4-amino quinoline (chloroquine) and 9-amino acridine (atabrine) derivatives of spermidine function well in the model system, 8-amino quinolives (primaquine) and certain other heterocyclic compounds containing spermidine substructures have no activity. The amount of polyamine necessary to reverse inhibition rises as the length of the poly A segment is increased. Thus, the protection rendered to the rest of the molecule by 3' terminal poly A induced inhibition of RNase activity appears to be quite general and may be regulated effectively by the following factors: shortening of the polyadenylated portion, changes in ionic strength, or alternations in the concentrations of either RNase or certain polyamines.

Publications:

Karpetsky, T.P., Hieter, P.A., Frank, J.J. and Levy, C.C.: Polyamines, Ribonucleases, and the Stability of RNA. Mol. and Cell. Biochem., in press, 1977.

Karpetsky, T.P., Boguski, M. and Levy, C.C. Polyadenylic Acid and Ribonucleases. Subcell. Biochem, in press, 1977.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 06933-02 LMB

PERIOD COVERED
July 1, 1976, to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Influence of Renal Insufficiency on Levels of Human Serum Ribonuclease

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	T.P. Karpetsky	Staff Fellow	LMB, NCI
OTHER:	C.C. Levy	Chief	LMB, NCI
	E.A. Neuwelt	Clinical Associate	LMB, NCI
	R.L. Humphrey	Staff	Johns Hopkins University Medical School

COOPERATING UNITS (if any)

Johns Hopkins Hospital
Oncology Department

LAB/BRANCH
Laboratory of Molecular Biology

SECTION

INSTITUTE AND LOCATION
NCI, NIH, Baltimore, Maryland 21211

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.8	0.4	0.4

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline key

The activity of serum ribonuclease (RNase) was measured in 29 patients with various types of leukemia and differing degrees of renal insufficiency. RNase levels are not a biomarker for the extent of leukemia. However, a strong inverse correlation was observed between the degree of renal impairment (expressed as creatinine clearance) and serum RNase levels. Time course studies of eighteen leukemia patients also indicated that RNase levels reflect the status of renal function and not the extent of leukemia. Levels of this enzyme, therefore, are not of use in determining the response of neoplasia to treatment. Enzyme concentrations, however, are useful in determining the degree of renal insufficiency and should prove particularly useful in cases where 24 hour urine collections are difficult, or where a creatinine deficit is suspected. The enzyme responsible for enhanced RNase activity as glomerular filtration rate declines is shown to be antigenically similar, if not idential, to the enzyme found in normal human plasma. At present, determinations of serum RNase levels in patients with Lupus Erytheinatosis are in progress, with the intent of proving that the elevations in enzyme concentrations reflect renal status and are not a biomarker for the disease itself.

Project Description:

The purpose of this study was to examine the utility of serum RNase levels as a biomarker for the presence and response to therapy of leukemia. Accordingly, RNase levels were measured in 29 patients with leukemia at various times during their illness and compared with 54 normal controls and 28 non-leukemic patients on chronic hemodialysis. Eighty-three percent of the leukemic patients examined before therapy and with normal rates of glomerular filtrations (as defined by creatinine clearance) greater than 60 ml/min had RNase levels that fell within the mean $\pm 2\sigma$ for the normal controls. Sixty-one percent of the leukemic patients, studied at various times during the course of their illness, who had a creatinine clearance of greater than 60 ml/min had RNase levels in the normal range. Comparison of RNase levels for leukemic patients who had not yet received treatment with those obtained from patients with leukemia having C_{CR} greater than 60 ml/min indicated that these two groups were statistically indistinguishable. Ninety-three percent of the leukemic patients studied with a creatinine clearance greater than 60 ml/min had RNase levels above the range established for the normal controls. Patients without leukemia, but on hemodialysis for chronic renal failure of varied etiologies, had markedly elevated serum RNase levels. A strong correlation between RNase levels and renal insufficiency is, therefore, demonstrated, illustrating that changes in RNase levels over periods of time depend on the rate of glomerular filtration and are not related to the extent of leukemia. The enzyme responsible for enhanced RNase activity as glomerular filtration rate declines is shown to be antigenically similar, if not identical, to the enzyme found in normal human plasma.

The overall findings of this study are that RNase concentrations are not a biomarker for leukemia, but are a indicator of renal function. These results are confined on examination of serum RNase levels in patients with forms of cancer other than leukemia (aplastic anemia, oatcell carcinoma, multiple myeloma). Studies are underway to determine the utility of measurements of RNase levels in cases where determinations of creatinine clearance are difficult or subject to error and in cases where a creatinine deficit may occur, resulting in falsely low values of serum creatinine. This study has focused on the measurement of levels of the major ribonuclease present in human serum. There are, however, several other ribonucleases present at levels of activity corresponding to less than one percent of this predominant activity. A sensitive and rapid gel electrophoresis method is under development in order to determine if levels of these other ribonucleases present in serum may be useful as biomarkers for multiple myeloma, leukemia or other types of neoplasia. It is also anticipated that examination of these RNase concentrations in serum from patients with various types of disorders, will also provide information concerning the tissues of origin of these enzymes.

Publications:

Karpetsky, T.P., Humphrey, R.L. and Levy, C.C.: Influence of Renal Insufficiency on Levels of Serum Ribonuclease in Patients with Multiple Myeloma. J. Natl. Cancer Institute 58: 875-880, 1977.

Humphrey, R.L., Karpetsky, T.P., Neuwelt, E.A. and Levy, C.C.: Levels of serum Ribonuclease as an Indicator of Renal Insufficiency in Patients with Leukemia. Cancer Research 37: 2015-2022, 1977.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 06963-01 LMB

PERIOD COVERED

July 1, 1976, to September 30, 1977

TITLE OF PROJECT (80 characters or less)

RNase Activity in Human Interferon Preparations

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	T.P. Karpetsky	Staff Fellow	LMB, NCI
OTHER:	C.C. Levy	Chief	LMB, NCI
	R.A. Winchurch	Staff Fellow	LMB ,NCI
	K. Cantrell	Central Public Health Laboratory Helsinki, Finland	

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Molecular Biology

SECTION

INSTITUTE AND LOCATION

NCI, NIH, Baltimore, Maryland 21211

TOTAL MANYEARS: 0.4	PROFESSIONAL: 0.4	OTHER: 0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline k

The level of ribonuclease (RNase) activity in human interferon preparations were examined. Although sequential purification of interferon resulted in nearly a 300 fold increase in specific activity. RNase specific activity remained more or less constant. These results indicate that although interferon can be separated from the great majority of contaminating proteins, RNase concentrations are not decreased during the course of the purification. Thus, levels of this enzyme contaminate even the highest specific activity interferon preparations. The large increase in the ratio of interferon to RNase activities concomitant with interferon purification indicates that these activities do not reside in the same protein, i.e., interferon does not have RNase activity.

PHS-6040
(Rev. 10-76)

Project Description:

Interferon (IF) has been reported to block translation of both cellular and viral messenger RNA in several systems. These results would seem to indicate that IF may be implicated in the loss of structural integrity of mRNA. We, therefore, sought to examine the effects of partially purified human foreskin fibroblast IF preparations on cellular RNase activity. These early studies revealed that treatment of radiolabelled RNA substrate with IF alone resulted in large increases in soluble radioactivity indicative of RNase activity in the IF preparation. Subsequently, examination of crude human leukocyte IF preparations (including reference samples obtained from the Research Resources Branch, National Institute of Allergy and Infestious Diseases, Rockville, Maryland) revealed the presence of high levels of RNase activity. Therefore, we sought to examine the relationship between IF activity and RNase activity by testing the levels of RNase in preparations of human leukocyte IF that had been sequentially purified to yield IF of increasing specific activity. At successive stages of purification, the specific activity of interferon increases, whereas that of RNase remains more or less stationary. Therefore, the ratio of interferon activity to RNase activity generally increases. However, concentrations of RNase contaminate even the most highly purified interferon preparations. These findings indicate that data derived from experiments in which exogenous interferon preparations are utilized must be critically analyzed, in view of the fact that exogenous RNase may be introduced along with the interferon. Thus, the mode of action of interferon inferred from translational studies cannot be accurately assessed in cell-free systems if interferon contains levels of RNase sufficient to degrade mRNA.

Publications:

Winchurch, R.A., Karpetsky, T.P., Levy, C.C. and Cantrell, K.: RNase Activity in Human Interferon Preparations. J. Virol. 21: 1247-1248, 1977.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 06964-01 LMB

PERIOD COVERED

July 1, 1976, to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Regulation of Messenger Ribonuclease and Stability in Mammalian Tissue

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	C.C. Levy	Chief	LMB, NCI
OTHER:	Gregory Davies	Staff Associate	LMB, NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Molecular Biology

SECTION

INSTITUTE AND LOCATION

NCI, NIH, Baltimore, Maryland 21211

TOTAL MANYEARS: 1.0	PROFESSIONAL: 1.0	OTHER: 0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline ke

Several endoribonucleases have been isolated from bovine skeletal muscle. The enzymes will be used to examine stability of messenger RNA mediated by polyadenylic acid inhibition of nucleolytic digestion.

PHS-6040
(Rev. 10-76)

Prior work in the laboratory has shown that ribonucleic acid can, under certain conditions, be protected against endonucleolytic degradation by the presence of polyadenylic acid at the 3' terminus. This may be of considerable physiologic importance since messenger ribonucleic acid from many different organisms has a strand of polyadenylic acid at the 3' end. The current work has shown that at least three endonucleases all of which are inhibited by polyadenylic acid, can be isolated from bovine skeletal muscle. These enzymes probably have different specificities for nucleotides in ribonucleic acid and may physiologically act to degrade different kinds of ribonucleic acid in the cell. Each enzyme will be examined for its ability to degrade messenger ribonucleic acid from muscle and for the ability of polyadenylic acid at the 3' end to inhibit this degradation. The findings may lend support to the above mentioned hypothesis concerning the function of polyadenylic acid *in vivo.* Regulation of nucleic acid metabolism is presumably deranged in neoplasia, and further knowledge of the mechanism by which messenger ribonucleic acid is stabilized in the cell could lend to conclusions about the differences between normal and neoplastic cells in this regard.

In addition to the problem of messenger ribonucleic acid stability, work on sequence determination of nucleic acids may benefit from isolation of several new endoribonucleases. The enzymes from bovine muscle differ in specificity: one enzyme degrades polycytidylic acid better than it degrades ribonucleic acid, while another does not attack polycytidylic acid at all but does attack ribonucleic or polyuridylic acid. Nucleases specific for certain residues in nucleic acids have been extensively used in sequence determination, it is hoped that knowledge of the base sequence in, for example, tumor viruses may contribute to elucidation of the mechanism of neoplasia. Bacterial endoribonucleases of useful specificity have been previously isolated in the laboratory and used by other workers in sequence determination.

Publications:

Hieter, P.A., LeGrendre, S.M. and Levy, C.C. Stabilization of an RNA molecule by 3' Terminal Poly A Induced Inhibition of RNase Activity. *J. Biol. Chem.* 251: 3287-3293, 1976.

Levy, C.C. Roles of RNases in Cellular Regulatory Mechanisms (Minireview) *Life Sciences* 17: 311-316, 1975.

Levy, C.C., Schmukler, M., Frank, J.J., Karpetsky, T.P., Jewett, P.B., Hieter, P.A., LeGrendre, S.M. and Dorr, R.G. Possible Role for Poly A as an Inhibitor of Endonuclease Activity in Eukaroytic Cells. *Nature* 256: 340-342, 1975.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 06965-01 LMB

PERIOD COVERED

July 1, 1976, to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Analysis of DNA Damage in vivo by Alkylating Agents and Ionizing Radiation

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	John Hilton	Visiting Associate	LMB, NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Molecular Biology

SECTION

INSTITUTE AND LOCATION

NCI, NIH, Baltimore, Maryland 21211

TOTAL MANYEARS: 1.4	PROFESSIONAL: 0.6	OTHER: 0.8

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline ke

A modification of the alkaline elution method of the analysis of single-strand breaks and cross-links in DNA allows the universal application of the method to tissues containing unlabelled DNA. DNA damage by multifunctional alkylating agents and ionizing radiation has been demonstrated in rat brain, brain tumor, liver, kidney, muscle and small intestine.

PHS-6040
(Rev. 10-76)

The alkaline elution method of analysis of single-strand breaks and cross-links in DNA has been modified to allow its universal application by removing the requirement for the use of radioactively labelled DNA. The alkaline eluted is dialyzed by a hollow fiber device to remove chemical co-precipitants prior to the acid precipitation of DNA. The amount of eluted DNA is measured by a sensitive fluorimetric assay. The results obtained by our modified method are identical to those obtained using radioactively labelled DNA.

Preliminary results indicate that the DNA of any tissue can be analysed for chemical or radiation induced single strand breaks and DNA cross-links. Cross-linking by a number of multifunctional alkylating agents (HN2, dianhydrogalactitol, spirohydantoin mustard, aziridinyl benzoquinones) and nitrosoureas (BCNU, CCNU) has been demonstrated in one or more of the following rat tissues: brain, brain tumor, liver, kidney, small intestine and bone marrow.

It is anticipated that gross observations of the chemotherapeutic action, pharmacology and toxicology of anti-tumor alkylating agents can be related to the extent of DNA damage and its repair in the target tumor and host tissues.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 06966-01 LMB

PERIOD COVERED
July 1, 1976, to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Enzymes of mRNA Processing and Degradation in Avian Liver

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	W.V.V. Greenhouse	Staff Fellow	LMB, NCI
OTHER:	C.C. Levy	Chief	LMB, NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Molecular Biology

SECTION

INSTITUTE AND LOCATION
NCI, NIH, Baltimore, Maryland 21211

TOTAL MANYEARS: 1.5	PROFESSIONAL: 1.0	OTHER: 0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this work was to isolate and characterize the enzyme(s) responsible for processing and eventual degradation of mRNA in avian liver in order to more fully understand the regulatory control mechanism of mRNA levels in eukaroytic cells. An RNase was purified 40,000 fold from chicken liver and its enzymatic properties studied. The enzyme was found to be strongly inhibited by polyadenylic and polyguanylic acids. This inhibition could be reversed by polyamines.

PHS-6040
(Rev. 10-76)

The purpose of this investigation was to study the regulatory mechanism responsible for the stabilization and eventual degradation of mRNA in avian liver.

A ribonuclease has been purified 40,000 fold from chicken liver homogenates and its enzymatic properties studied. It appears to act as an endoribonuclease which produces cyclic 2',3'-nucleotides but does not open the cyclic 2',3' ring. Of special interest is inhibition of this enzyme by poly A and poly G. The inhibition is competitive; poly G is a significantly stronger inhibitor than poly A. Polyamines greatly stimulate enzyme activity at low ionic strength. Also, these compounds can reverse the inhibition of poly A or poly G at low ionic strength. The properties allow for a regulatable stabilization of mRNAs via the poly A sequence at the 3' terminus of the mRNA.

Future studies will examine the interaction of this enzyme with mRNA extracted from chicken liver and the nature of the control of mRNA degradation. Further, the specificity of the enzyme for internucleotide linkages will be examined with the purpose of investigating its usefulness as an enzymatic tool in the sequencing of mRNAs.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM06967-01 LMB

PERIOD COVERED
July 1, 1976, to September 30, 1977

TITLE OF PROJECT (80 characters or less)

The Study of the Factors Involved in Stabilization of Amphibian mRNA

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	C.C. Levy	Chief	LMB, NCI
OTHER:	M.S. Boguski	Research Assistant	LMB, NCI
	K.P. Appich	Research Assistant	LMB, NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Molecular Biology

SECTION

INSTITUTE AND LOCATION
NCI, NIH, Baltimore, Maryland 21211

TOTAL MANYEARS: 0.8	PROFESSIONAL: 0.4	OTHER: 0.4

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of the study was to establish the factors responsible for the overall metabolism of mRNA isolated from the African toad, Xenopus laevis.

PHS-6040
(Rev. 10-76)

Project Description:

At the 3' terminus of mRNA is a chain of adenylic acid residues (poly A), the function of which is not understood. Observations in this laboratory have shown that polyadenylic acid is a competitive inhibitor of a number of ribonuclease. It can be shown, in fact, taht the polynucleotide and the protein bind to form a complex which can be isolated and characterised. In an attempt to examine a variety of tissues to test the hypothesis that poly A stabilizes mRNA by inhibiting ribonuclease activity, the RNase from Xenopus laevis liver has been isolated. The enzyme has been purified to homogenity and has a specific activity of 60,000 units per mg of enzyme protein. The enzyme has a requirement for magnesium and shows a proclivity for uridylic acid residues at the 3' terminus bot no particular specificity at the 5' terminus of the phosphodiester bond. Inhibition occurs by polyadenylic acid with an inhibitory constant of 2×10^{-6} M. Vitellogenesis mRNA from Xenopus liver can be degraded by this enzyme if poly A at the 3' terminus of mRNA is removed. In the absence of poly A, no translation of mRNA occurs, whereas if the terminal tract of poly A is retained, the protein formed appears to be immunologically the same as vitellogenin isolated from Xenopus liver.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 06020-09 LMB

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)
Mechanism of Human Lymphocyte Reactivity to Non-Self

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	M.R. Mardiney, Jr.	Head, Immunology and Cell Biology	LPH NCI
Other:	R.A. Winchurch	Staff Fellow	LPH NCI
	S. Shu	Staff Fellow	LPH NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH Laboratory of Molecular Biology

SECTION Immunology and Cell Biology

INSTITUTE AND LOCATION
NCI, NIH , Baltimore, Maryland 21211

TOTAL MANYEARS: 2	PROFESSIONAL: 1.5	OTHER: .5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline k

An intensive program has been followed to characterize the sequence and mechanism of human lymphocyte responsiveness to specific antigen in vitro within the blastogenesis system. Towards this end, development and application of automated laser cytometry to this assay was carried out. Laser cytometry was found to allow for a fuller characterization of the nature of immune responsiveness within this assay of cell associated immunity. In addition, the biologic effect of hormonal agents [adrenergic stimulators (agonists) and blockers] on antigen stimulated immune response was studied.

Project Description:

As a first step, the analysis of alteration of laser light transmission by single, viable, unstained cells during the course of lymphocyte blastogenesis assays was carried out. The use of a monochromatic collimated light source focused by a cylindrical lens to a narrow elipse results in photometric illumination distributed in sharply peaked Gaussian distribution along the axis of cell flow. The present methodology allowed for measurements of changes in the laser beam which occur secondary to the intersection of the light source by a cell. Changes in laser beam transmission were detected by photosensors and the information processed by a multichannel analyzer to obtain a 100 channel pulse height analysis which was displayed, printed and stored. Laser analysis of human lymphocyte responsiveness was compared to the standard ^{3}H thymidine assay as well as actual morphologic analysis of lymphocytes at various stages of in vitro responsiveness. It was ascertained that cellular changes during the blastogenesis response could be rapidly ascertained by laser cytometry. The previous discordance noted between actual lymphocyte proliferation and thymidine incorporation on exposure to specific antigen or allogeneic cells was reaffirmed. High doses of antigen or allogeneic cells which were found to diminish thymidine incorporation were not associated with actual diminishment in lymphocyte proliferation but were found to enhance it. In addition, the sequence of changes within the system indicated that a significant number of small lymphocytes were produced in this system and that these small lymphocytes reverted back to lymphoblasts in cyclical fashion. Hormonal studies revealed that the presence of beta blockers, propranolol and dichloroisoproterenol enhanced the antigen induced response and that this effect appeared to be related to specific blocking of the lymphocyte beta receptor since D (+) propranolol, a compound devoid of such activity, had no effect.

Publications:

Ruckdeschel, J.C., Doukas, J.G., Drake, W.P., and Mardiney, M.R., Jr.: Application of Laser Cytometry to the Analysis of Immunologically Induced In Vitro Lymphocyte Responsiveness. Transplantation 23: 396-403, 1977.

Doukas, J.G., Ruckdeschel, J.C., and Mardiney, M.R., Jr.: Quantitative and Qualitative Analysis of Human Lymphocyte Proliferation to Specific Antigen in Vitro by Use of the Helium Neon Laser. J. Immunol. Methods 15: 229-238, 1977.

Winchurch, R.A., and Mardiney, M.R., Jr.: The Effects of Adrenergic Agonists and Blockers on Antigen-Induced DNA Synthesis in Vitro. Biomedicine 26: 36-42, 1977.

Winchurch, R.A., and Mardiney, M.R., Jr.: In Vitro Discordance between Actual Lymphocyte Proliferation and Thymidine Incorporation Upon Exposure of Human Lymphocytes to Specific Antigen or Allogeneic Cells. Transplan. Proc. 9: 873-876, 1977.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 06292-03 LMB

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Immune Adjuvants

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	M.R. Mardiney, Jr.	Head, Immunology and Cell Biology	LPH NCI
Other:	R. A. Winchurch	Staff Fellow	LPH NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Molecular Biology

SECTION
Immunology and Cell Biology

INSTITUTE AND LOCATION
NCI, NIH, Baltimore, Maryland 21211

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1	1/2	1/2

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline k

Surgery, a major mode of cancer therapy, has been hampered by the unanticipated occurrence of cancer generally due to microscopic foci of tumor not originally known. As a result, combined modality treatment has been increasingly utilized to alter the natural history of recurrence of neoplasia despite extensive surgery. With this background, the therapeutic effectiveness of surgery, chemotherapy and immunotherapy alone and in combination was studied within the B16 melanoma model. In addition, because of past experience indicating the effectiveness of poly A:U in prolonging survival of AKR mice, experiments were carried out to compare the adjuvant properties of various synthetic polynucleotides upon the response of sensitized human lymphocytes in vitro to specific antigen.

Project Description:

Within the B16 melanoma model, C57BL/6J mice were given 1 x 10^5 viable B16 melanoma cells injected into the left hind limb just above the knee on day 1 and randomly divided into 16 groups. Group 1 served as untreated controls. The remaining groups were treated with surgery, chemotherapy or immunotherapy alone or in combination. Chemotherapy consisted of cyclophosphamide 250 mg/kilogram; immunotherapy consisted of 0.4 mg of BCG (Phipps strain), and surgery was performed by means of a hot cautery. In order to determine the results of biologic variation, the entire experiment was performed three different times. Within the first experiment, each group consisted of 20 mice while in the second and third experiments ten mice each. The sequence of chemotherapy and immunotherapy as adjuvants to surgery proved important. Chemotherapy was significantly better when given before surgery, immunotherapy was more effective when delivered after surgery. The most effective therapeutic regimen was a combination of all three modalities: a single course of chemotherapy preceding surgery followed by immunotherapy. This combination of treatment consistently provided the longest median and mean survival times. In in vitro experiments, the comparative effects of poly A:U, poly G:C and poly I:C upon the response of human sensitized lymphocytes to specific antigen was studied. It was demonstrated that all three synthetic polyribonucleotides were capable of amplifying antigen induced in vitro ^{3}H thymidine incorporation of human lymphocytes in response to specific antigen.

Publications:

Pendergrast, W.J., Jr., Drake, W.P., and Mardiney, M.R., Jr.: A Proper Sequence for the Treatment of B16 Melanoma: Chemotherapy, Surgery and Immunotherapy. J. Natl. Cancer Inst. 57: 439-544, 1976.

Pendergrast, W.J., Jr., Futrell, W.J., and Mardiney, M.R., Jr.: Differences in Potentiation of Melanoma Growth by Absorbable and Non-Absorbable Sutures. J. Surg. Oncology 8:223-228, 1976.

Winchurch, R.A., Godla, G., Birmingham, W., and Mardiney, M.R., Jr.: Comparative Effects of Different Polynucleotide Complexes on the Immune Response of Human Peripheral Blood Lymphocytes. Transplantation 21: 423-426, 1976.

Graziano, K.D., and Mardiney, M.R.,Jr.: The Synergistic Effect of Synthetic Polynucleotides and Mercaptoethanol in Amplifying the Murine Mixed Lymphocyte Reaction. Transplantation 21: 317-322, 1976.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF** **INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 06932-01 LMB

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)
Correlation of Abnormal Nuclease Activity to the Prediction and Presence of Cancer

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	M.R. Mardiney, Jr.	Head, Immunology and Cell Biology	LPH NCI
Other:	D. Maor	Visiting Scientist	LPH NCI

COOPERATING UNITS (if any)
Tumor Immunology Branch, Surgery Branch, NCI, NIH, Bethesda, Maryland 20014

LAB/BRANCH
Laboratory of Molecular Biology

SECTION
Immunology and Cell Biology

INSTITUTE AND LOCATION
NCI, NIH, Baltimore , Maryland 21211

TOTAL MANYEARS: 2	PROFESSIONAL: 1	OTHER: 1

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline ke

The association of elevated nucleolytic activity with the cancer and precancer state was further studied by analyzing serum ribonuclease activity in 11 different human cancers utilizing as substrates a variety of synthetic polynucleotides. The finding of significant elevation in all 11 cancer states provides additional evidence for the association of elevated nucleolytic activity and neoplasia.

Project Description:

Alkaline serum ribonuclease activity of 500 cancer patients and 168 healthy donors were carried out and analyzed. Maximum serum ribonuclease activity occurred at pH 7.5 and although most of the alkaline serum ribonuclease activity was directed against poly C, changes in activity were reflected independently against other synthetic polynucleotides. Three substrates were utilized in such assays - poly C, poly U and poly A·poly U. Mean serum ribonuclease activity in 168 healthy donors was 36 units/ml (S.D. 9.8 units/ml) against poly U, 850 units/ml (S.D. 248 units/ml) against poly C and 3.9 units/ml (S.D. 0.8 units/ml) against poly A·poly U. The upper limit of "normal" for serum ribonuclease levels was fixed at two standard deviations above the mean of healthy donors. Levels greater than two standard deviations from the mean were considered abnormally elevated. Eleven malignancies were studied: acute non-lymphocytic leukemia, brain tumor, adenocarcinoma of the breast, cervical carcinoma, chronic myelocytic leukemia, colorectal carcinoma, Hodgkin's disease, carcinoma of the lung, non-Hodgkin's lymphoma, pancreatic carcinoma, and sarcoma. The pronounced phenomenon observed was the significant difference ($p<0.001$) between the 11 groups of cancer patients and between the group of normal controls against each of the three synthetic substrates utilized in assaying ribonuclease activity. Analyzing serum ribonuclease activity in every individual showed that a serum sample could exhibit increased activity against one, two or all three of the substrates. Abnormally elevated activity against one substrate did not necessarily indicate elevated activity against all. 78% or more of the cancer patients of nine malignancies studied had elevated serum ribonuclease activity against at least one or more of the three substrates (acute non-lymphocytic leukemia, chronic myelocytic leukemia, non-Hodgkin's lymphoma, brain tumor, cervical carcinoma, colorectal carcinoma, carcinoma of the lung, pancreatic carcinoma, and disease). 61% of patients with breast carcinoma, and 60% of patients with sarcoma demonstrated elevation against one or more substrates.

Publications:

None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 06968-01 LMB

PERIOD COVERED
July 1, 1977 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Cell Associated Immunity to Viruses

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	M.R. Mardiney, Jr.	Head, Immunology and Cell Biology	LPH NCI
Other:	Margaret Pass	Research Biologist	LPH NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Molecular Biology

SECTION
Immunology and Cell Biology

INSTITUTE AND LOCATION
NCI, NIH, Baltimore, Maryland 21211

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1/4	1/8	1/8

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Since lymphocyte responsiveness within the blastogenesis system is primarily a memory dependent effect and is not dependent upon the presence of specific antibody it was felt that this in vitro assay of cellular immunity might be a sensitive indicator of prior exposure to viral infection and could be utilized as a method of determining susceptibility to specific viral disease. Because of the importance of the herpes viruses as a source of recurrent disease in man and because of their association with the oncogenic state, the characteristics of human lymphocyte responsiveness to these viruses were studied.

Project Description:

As a first step, the nature of normal human lymphocyte responsiveness to a crude varicella zoster antigen was studied. Varicella zoster antigen was obtained from human foreskin cells (MA/184) infected with varicella zoster virus in the absence of serum. Infected cells obtained on day 5 were sonicated and a membrane rich supernate obtained following low speed centrifugation was utilized as the antigen source. A mock viral control consisted of uninfected cells processed similarly. Electron microscopy evaluation of these two preparations revealed them to contain many cell membrane fragments. The varicella zoster preparation was noted to contain whole herpes virions. Such a varicella zoster antigen preparation was shown to elicit specific lymphocyte responsiveness in all normal adults studied. Such responsiveness occurred even in those individuals lacking circulating antibody to varicella zoster antigen. Because of the known impaired cell associated immunity to varicella zoster virus in patients with Hodgkin's disease, 32 patients with this disease and 12 normal donors were studied for their in vitro lymphocyte responsiveness to this antigen. When compared to normal donors, patients with Hodgkin's disease in whom radiotherapy was recently completed and those with active, recurrent disease had markedly impaired cell associated immunity to varicella zoster antigen. In addition, there was a suggestion that patients in long-term remission who had received primary combined modality therapy (radiotherapy + chemotherapy) had an impaired response when compared to normal persons or to patients who had received single modality therapy. Newly diagnosed, untreated patients with Hodgkin's disease did not differ significantly from normal persons as a group but two of six were unresponsive to the varicella zoster antigen whereas all normal subjects, as noted above, were responsive. Most patients in remission for at least one year following therapy had normal in vitro responsiveness. In two patients herpes zoster developed after the demonstration of absent in vitro lymphocyte reactivity to the varicella zoster antigen suggesting that the initial absence of responsiveness in this patient population might predict those at highest risk for developing this disease.

Publications:

Ruckdeschel, J.C., Schimpff, S.C., Smyth, A.C., and Mardiney, M.R., Jr.: Herpes Zoster and Impaired Cell-Associated Immunity to the Varicella-Zoster Virus in Patients with Hodgkin's Disease. Amer. J. Med. 62: 77-85, 1977.

Mardiney, M.R., Jr., and Ruckdeschel, J.C.: Discussion - Inhibition of in Vitro Lymphocyte Responsiveness by Measles Virus Antigen. Cell. Immunol. 28: 229, 1977.

Ruckdeschel, J.C., and Mardiney, M.R., Jr.: The Demonstration of Cell Associated Immunity to Viruses: In Vitro Lymphocyte Responsiveness to Varicella Zoster Antigen. J. Immunol. Methods 13: 131-143, 1976.

ANNUAL REPORT
OFFICE OF THE ASSOCIATE DIRECTOR
CANCER THERAPY EVALUATION PROGRAM
DIVISION OF CANCER TREATMENT
NATIONAL CANCER INSTITUTE

The Cancer Therapy Evaluation Program (CTEP) may be considered to have an intramural function as the clinical arm of the Division of Cancer Treatment's Developmental Therapeutic Program and an extramural function in sponsoring, monitoring, and evaluating clinical trials directly relevant to cancer treatment. It is responsible for: 1) coordinating the introduction of new anticancer agents into clinical trials (Investigational Drug Branch [IDB]), 2) providing the necessary feedback from clinical results to the Developmental Therapeutics Program (all branches), 3) monitoring the evaluation of such agents by contract (IDB and Combined Modality Branch [CMB]) and grant supported (Clinical Investigations Branch [CIB]) groups, 4) integrating therapeutic modalities under investigation with other established therapeutic modalities in the treatment of advanced and early cancer of various sites (CMB), and 5) assisting the Director of the Division of Cancer Treatment and the Assistant Director for International Treatment Research in representing the NCI in international agreements dealing with exchange on clinical trials (Office of the Associate Director).

All the activities of the three Branches in the CTEP are integrated by the Associate Director, CTEP for the purposes of 1) centralizing and updating information on cancer treatment, 2) bringing together the drug-oriented and disease-oriented thrusts of the Program, 3) introducing new modalities ready for implementation into clincial trials, and 4) developing strategies for the treatment of various neoplastic diseases. The office of the Associate Director, CTEP, is in an ideal position to stimulate therapeutic research along avenues believed most productive. Both contract and grant programs aid in carrying out this research and will be described under the respective Branches. Table 1 indicates the procedures that are followed to pursue new therapeutic leads. The organizational structure to implement such procedures is described here to include the following functions:

- I. Data center for DCT supported clinical trials
- II. Analysis of new drug information
- III. Development of disease-oriented strategy
- IV. Standardization of clinical trials methodology
- V. Coordination with other clinical trials:
    - a. Intramural
    - b. DCT international activities
    - c. Other
- VI. Training

All personnel in the CTEP contribute to these functions; the

Table 1. Serial Procedures in Pursuing New Therapeutic Leads.

1. In staff review:
    a. Literature search
    b. Current disease oriented studies (grantees, contractors, other)
    c. new drug information (clinical and preclinical)

2. Review of ongoing intramural grant and contract supported studies: Cooperative Discussion Group

3. Assemble working study group for recommendations on new directions

4. Designate protocol writing committee from study group, when appropriate

5. Present plans to Board of Scientific Counselors, DCT

6. Issue RFP or encourage grant supplement application from cooperative groups

7. Review of new projects by appropriate peer-technical review committees

specific personnel assigned to coordinate them within the office of the AD will be identified when appropriate.

I. Data Center for DCT Supported Clinical Trials

The centralization of major contract and grant treatment thrusts under one program has provided the opportunity to develop a data center including most ongoing treatment-related research. A protocol file, including results of all current DCT-supported trials, has been established since May 1976, under the direction of Ms. Elise Mackie and maintained by Mrs. Linda Edge. These files have been used by members of DCT in preparation of new protocols, as well as scholarly reviews of ongoing work. Information of the Data Center has also been used to help prepare the yearly NIH inventory of clinical trials.

The Data Center has been set up by close interfacing with the International Cancer Research Data Bank (ICRDB). It has assisted in the development of abstracts of all clinical protocols to be included in a registry to be published by the ICRDB, as well as appearing in the computer retrievable CLINPROT file (National Library of Medicine computer) including now more than 900 entries. Updating of the registry and abstracts is worked out by close coordination between Ms. Mackie and Mr. Richard Amacher, Office of the Director, NCI responsible for the ICRDB, and the Smithsonian Scientific Information Exchange. Although the CTEP Data Center maintains an updated file primarily of all DCT-supported studies, it also provides information and guidance in the abstracting of other clinical trials (such as other grant-supported studies, intramural studies, task forces, Cancer Center studies, international group and institutional studies). This coordinating function will be described more later.

Access to online ICRDB registry (CLINPROT) and Cancer Line, is available in the Data Center.

II. Analysis of New Drug Information

Responsibility for monitoring information on new drugs under sponsorship by the DCT lies within the IDB. Information is analyzed and provided on an annual basis to the FDA. In the office of the AD, CTEP, certain functions on new drug information are centralized:

A) Feedback to Developmental Therapeutics Program (DTP): A major effort is made to provide clinical input into the DTP to guide future drug development and to help assess the quality and need of preclinical work being carried out. For example, combined radiation and drug animal tumor studies rely on our Program for selecting drugs of clinical interest in evaluating this interaction. Another major area of input is in selection of analogs and assessing problems related to their testing. Dr. Marcel Rozencweig has initiated an analysis of comparative clinical studies with analogs. Various members of the CTEP have contributed to analog committees including the actinomycins, nitrosourea and alkylating agents, anthracyclines, platinum compounds, and vinca alkaloids.

B) Integration of information in disease-oriented strategy: Information from the Phase I and II monitored by the IDB is made available in the biweekly meetings of Protocol Review Committee, as part of a disease-oriented strategy (see below).

C) Adverse drug reactions: All reactions are rapidly analyzed by a committee with representation from all branches of the CTEP (Figure 1). Provisions are made for input from other areas of the Program such as the Pharmaceutical Resources Branch as needed.

III. Development of Disease Oriented Strategy

Personnel from the three branches and occasionally elsewhere within the Division of Cancer Treatment serve as disease-oriented coordinators (Table 2). One or more people are involved in monitoring various aspects of a disease and the activities sponsored by the NCI. In general, an interested representative from the Investigational Drug Branch forms a part of a team of coordinators. Usually the coordinator(s) is project officer on contracts dealing with the respective disease, and attends all meetings of the contract-related group as well as meetings pertinent to his area, such as various task forces, disease-oriented committees within cooperative groups, intramural programs, etc. Coordinators are then in ideal positions to serve as reviewers for protocol studies and to help develop strategy outlines.

Disease-oriented strategies have been developed for lung cancer, breast cancer, colorectal cancer, melanoma and sarcomas, which include priorities required for testing at each stage of disease. Similar plans are being developed for other diseases. Flow-charts included in the Treatment Document summarize the overall therapeutic trends and questions.

Cooperative discussion groups, assembled by the CTEP, either through the AD office or through the CIB, have been instrumental in guiding the cooperative groups and contractors to evolve therapeutic strategies. Additional details of some of these are given in the CIB Annual Report. Since the FY 1976 Annual Report, discussion groups on non-Hodgkin's lymphoma, adult leukemias, pediatric solid tumors, testicular carcinoma, melanoma, osteogenic sarcoma, bladder carcinoma, and adjuvant colorectal carcinoma have been assembled. In addition, working groups on specific aspects of clinical trials on endometrial cancer, testicular cancer, and head and neck cancer, met. The Lung Cancer Working Group convened once and advised on surgical adjuvant trials, which are expected to begin in July, 1977. The testicular cancer meeting is eventuating in an intergroup

FIGURE 1 FLOW CHART OF ADVERSE DRUG REACTION (ADR)

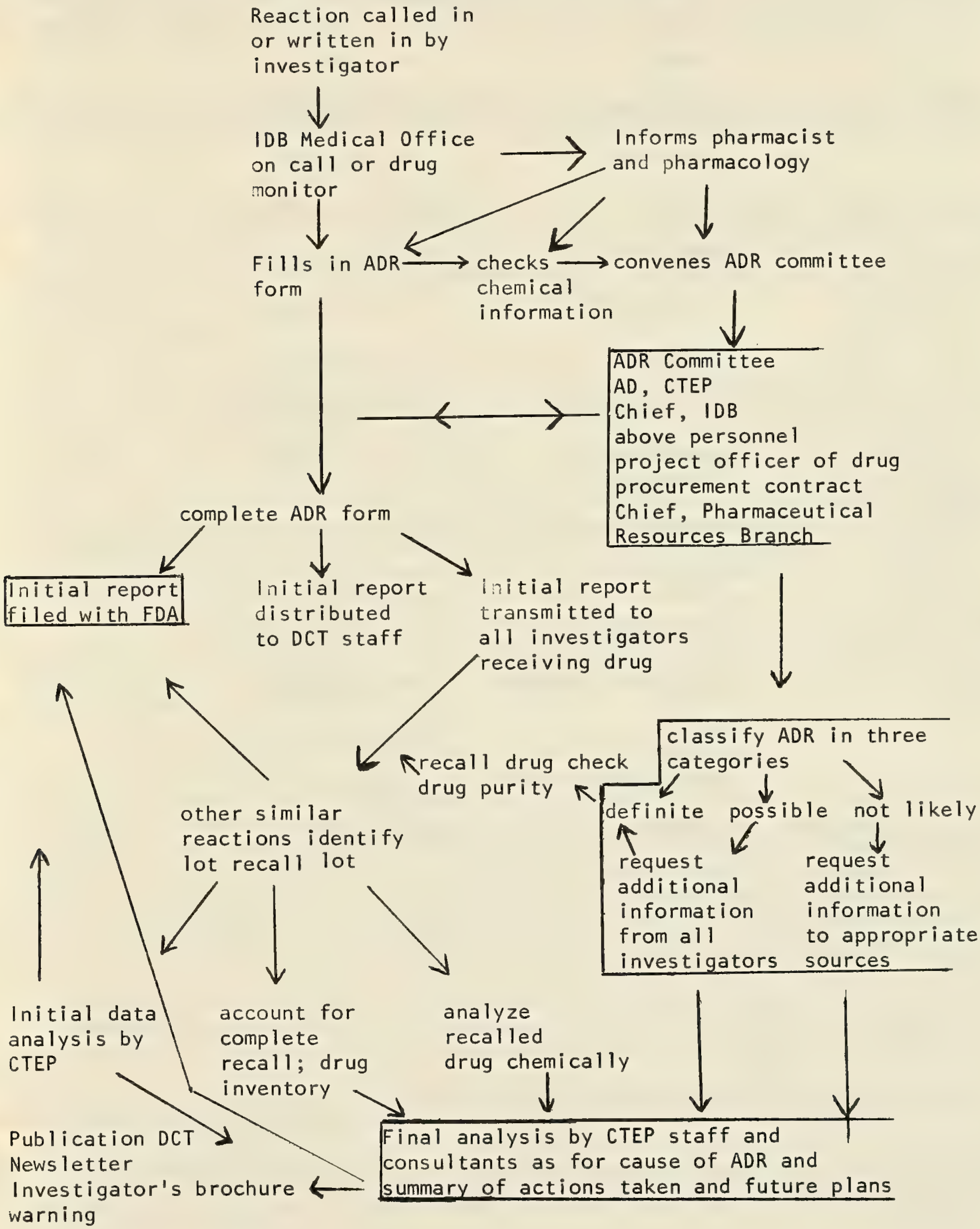
Reaction called in or written in by investigator
IDB Medical Office on call or drug monitor
Informs pharmacist and pharmacology
Fills in ADR form
checks chemical information
convenes ADR committee
ADR Committee
AD, CTEP
Chief, IDB
above personnel
project officer of drug procurement contract
Chief, Pharmaceutical Resources Branch
complete ADR form
Initial report filed with FDA
Initial report distributed to DCT staff
Initial report transmitted to all investigators receiving drug
recall drug check drug purity
classify ADR in three categories
definite
possible
not likely
other similar reactions identify lot recall lot
request additional information from all investigators
request additional information to appropriate sources
Initial data analysis by CTEP
account for complete recall; drug inventory
analyze recalled drug chemically
Final analysis by CTEP staff and consultants as for cause of ADR and summary of actions taken and future plans
Publication DCT Newsletter
Investigator's brochure warning

TABLE 2: DISEASE COORDINATORS

BREAST	DR. DAVIS
LYMPHOMA	DR. DAVIS
LEUKEMIA	DR. DAVIS
LUNG	DR. McGUIRE
OVARY	DR. McGUIRE
GYNECOLOGY	DR. McGUIRE
MELANOMA	DR. JACOBS
OSTEOSARCOMA	DR. HANDELSMAN
SOFT TISSUE SARCOMA	DR. JACOBS
HEAD & NECK	DR. CHRETIEN
GI	DR. HANDELSMAN
PROSTATE	DR. HANDELSMAN
BLADDER, KIDNEY	DR. McGUIRE
TESTIS	DR. JACOBS
CHILDHOOD TUMORS	DR. DAVIS
BRAIN	DR. McGUIRE

Stage II protocol. The head and neck cancer meetings resulted in an issuance of an RFP; this could be traced directly to the discussion group held March 25, 1976 and described in the FY 1976 Annual Report.

The Endometrial Cancer Study Group Meeting resulted in development of a protocol assessing hormone receptors by the Gynecology Oncology Group.

Minutes of the cooperative discussion groups have been assembled and disseminated widely to contractors, grantees and other investigators here and elsewhere. In addition, the papers presented at the Osteosarcoma Study Group, representing a summary of efforts spanning five years, will be published as a supplemental issue of Cancer Treatment Reports.

Finally, as an outgrowth of the Cooperative Discussion on Lung Cancer (November, 1975) it was also decided to review in detail the current trials at various stages of this disease. For this purpose a two-day meeting at Airlie House, Virginia was set aside taking place May 22-24, 1977. This meeting included more than 120 participants from various institutions and countries; the agenda is shown as an Appendix. A list of presented abstracts has been assembled and disseminated upon request. The formal presentations, with review of the status of previous trials carried out in lung cancer, will be published as a proceedings. It is expected that the deliberations emanating from such a meeting will point the way to future trials in both small cell and non-small cell lung cancer and has already led to agreements in modifying terminology and reporting of clinicopathologic findings.

IV. Standardization of Clinical Trials Methodology

A major effort of the CTEP will be in this direction. The need for standardization of criteria of entry, staging, response reporting and assessment, and end-results reporting is particularly apparent when attempting to coordinate and analyze treatment studies. Since many of these studies are coordinated via the CTEP, we are in an ideal position to recognize similarities and differences. A stepwise implementation of these procedures is envisioned:

A) Reconciliation of differences in definition of response as applicable to Phase II studies in lung, breast, colorectal cancers, sarcoma and melanoma (and later extending to others).

B) Characterization of methods of assessing these responses, including restaging.

C) Defining optimal eligibility criteria for Phase II advanced disease studies and for adjuvant or combined modality studies, respectively.

D) Ensuring that staging criteria and staging methods are those commonly defined and applied.

E) Encouraging accurate and well defined end-results reporting.

F) Development of master protocols for Phase II studies.

G) Computerization of data so collected.

V. Coordination with other Clinical Trials

It is essential that CTEP sponsored trials be coordinated with activities being carried out elsewhere. This coordination is partly achieved by encouraging processing by the Data Center of all treatment related trials. Moreover, some coordination has been formalized by representation of the CTEP in the intramural program of the DCT (Clinical Oncology Program [COP]) and in international agreements and activities.

A) Intramural Clinical Trials:

The AD participated in the Clinical Research Committee of the Clinical Center. In addition, studies utilizing new drugs by the Clinical Oncology Program Branches are carried out in concert with the IDB. Similarly the COP is a member of the Phase I Working Group.

Increasing cross-fertilization with the COP is envisioned by active participation of CTEP staff in clinical rounds and in other activities of the COP. This interaction will take place also at the level of staff associates. Ready implementation of new therapeutic leads and, conversely, prompt feedback of clinical studies to personnel concerned with its monitoring are obvious benefits of such close ties.

Specific preliminary exchanges have taken place between personnel in the Radiation Oncology Branch studies dealing with small cell carcinoma and Dr. Muggia. Dr. Von Hoff participated in activities of the Pediatric Oncology Branch dealing with agents in leukemia such as glutaminase and azacytidine and pharmacologic studies with platinum. Drs. Von Hoff and McGuire established continued communication with the Clinical Pharmacology Program and work on anthracycline cardiotoxicity. Dr. Delia Chiuten rotated through the Medical Oncology Branch of BCRC and then assisted in design of protocols with new agents. Dr. Alan Keller spent one year at the NCI-VA Medical Oncology Branch and helped develop and carry out protocols with high dose methotrexate and ifosfamide. Dr. Muggia assisted Dr. Chretien, of the Surgical Oncology Branch, in the design of high dose infusion methotrexate studies in head and neck cancer.

B) International Activities:

Collaboration with other countries takes place by means of international agreements, informal relationship with foreign cooperative groups, grant-supported activities of foreign institutes in our cooperative groups (details provided under CIB), contract-supported activities with specific institutes (details provided under CMB), and other relationships with foreign clinical cancer research programs carrying on studies with investigational drugs under our sponsorship, or of related interest.

The following countries are included in major activities in this area:

1. Belgium. The E.O.R.T.C. Data Center is supported by grant. The work includes linkage with other European participating countries in various E.O.R.T.C. cooperative groups. These studies count with the official support of the Belgian Ministry of Health, with liaison to other European countries.

2. Canada. Progress of studies sponsored by the NCI of Canada is followed closely by Dr. William McGuire. Scientific input, as well as access to investigational drugs for study, is provided on an ongoing basis. These studies include major national studies on Hodgkin's Disease, multiple myeloma, and ovarian cancer.

3. Egypt. Phase II studies in bladder cancer were initiated as part of U.S. - Egypt Agreement on Cancer Research and subsequently expanded to include work of the Cancer Institute of Cairo University with the Southwest Oncology Group and with the Clinical Oncology Program.

4. France. Phase II studies with diglycoaldehyde in acute leukemia (Dr. M. Boiron) and with cis-platinum in various solid tumors (Drs. G. Mathe and M. Hayat) have been initiated as part of a bilateral agreement.

5. Latin America. Through the Pan American Health Organization (PAHO), a program has been launched initiating exchange in protocol treatment studies between NCI and other U.S. institutes, with institutes within Argentina, Brazil, Chile, Colombia, and Peru. The scope of these programs will be apparent in the next Annual Report.

6. U.S.S.R. Exchange in chemotherapy has taken place since 1972. The Cancer Research Center (Moscow) and Petrov Institute (Leningrad) have carried out clinical studies with drugs sponsored by the NCI, with input from our Program. For example, studies have been initiated with CCNU in lung cancer, adriamycin and DTIC in small cell cancer of the lung, actinomycin D and DTIC in melanoma.

7. Japan. Via contract support, information on drug development and clinical studies is accrued. Conversely, the CTEP contributes clinical information to the Japanese Cancer Research Foundation of trials in the U.S.

8. Other. The CTEP has served as advisor to planned studies in breast cancer in Tunisia and to studies with investigational drugs in cancer centers in England, Poland, Hungary, Spain, South Africa, Lebanon, Italy, Israel, and to a number of other countries. Selected investigators in these countries have made outstanding contributions in the evaluation of antitumor drugs sponsored by the CTEP.

C) Other Clinical Trials:

The following task forces have had input from the CTEP, and vice versa, its activities have been monitored for close coordination.

1. Prostate - Dr. Handelsman attended as member of the Working Cadre.

2. Bladder - Dr. McGuire, together with Dr. Tom Anderson (COP), attended activities of this task force.

3. Breast - Dr. Muggia forms part of the Treatment Committee; many sessions have been attended by Drs. Davis and Jacobs.

4. Large Bowel - Drs. Handelsman and Muggia attended selected sessions of this Task Force.

Reassignments and coverage of all task forces including pancreas are anticipated for FY 1978.

clinical research within the U.S. Cancer Centers will be encouraged by making investigational drugs available to the centers to carry on special studies. For this purpose, drugs classified as Group B (drugs through initial Phase I testing) will be made available to Cancer Centers.

Finally, the CTEP maintains a dialogue with Clinical Research personnel of major pharmaceutical companies concerned with clinical trials, as well as other entities concerned with clinical trials such as local cooperative groups and foreign groups and international groups.

VI. Training.

Staff associates and visiting fellows are recruited for the IDB for two year periods: one year is spent in IDB work (clinical drug evaluation), and in the following year, individual arrangements have been made for clinical or laboratory rotations, or projects within other areas of the CTEP (clinical trials methodology; disease oriented strategies). Dr. Delia Chiuten was a visiting fellow and Drs. Arthur Louie and Alan Keller were staff associates during FY 1977.

Clinical Associates from the COP are encouraged to spend periods from three months to one year learning about clinical trials or aspects of clinical drug evaluation.

Guest workers spend from two weeks to three months learning about specific prearranged subjects within these broad designated areas. Drs. Hernan Cortes-Funes (Spain), Moise Namer (France), and Luis Riva (Peru) were guest workers during FY 1977.

VII. Publications

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35. Von Hoff, D.D., Rozencweig, M., and Muggia, F.M.: Cytembena: An antitumor drug with a unique structure. *Biomedicine* (in press).

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VIII. Presentations

Franco M. Muggia, M.D.

1. Current combined modality trials in breast, colon, and lung carcinomas, Perugia, Italy on July 2, 1976.
2. Presentation to the Head and Neck Advisory Group on August 5, 1976, in Bethesda, Maryland.
3. Methylnitrosourea. Nitrosourea Symposium, Bethesda, Maryland, September 9, 1976.
4. Development and evaluation of anticancer drugs. Japan, September, 1976.
5. Current programs of the cooperative groups. Non-Hodgkin's Lymphomas Symposium, San Francisco, CA, October 2, 1976.
6. Overview of status of investigational drugs. Phase I Working Group Meeting, Bethesda, MD, October 7, 1976.
7. Drugs from outside the NCI program. Phase I Working Group Meeting, Bethesda, MD, October 7, 1976.
8. Three presentations: 1) Roles in therapy: chemotherapy, 2) Training period required: In chemotherapy, and 3) Potential role of the Division of Cancer Treatment. Workshop on Training of Gastroenterologists in Cancer, Bethesda, MD, November 18-19, 1976.
9. Three presentations: 1) Current status of clinical trials - creation of data and safety monitoring boards, 2) Investigational drugs and the cooperative groups, and 3) Contract RFP's and the cooperative groups. Cooperative Group Chairmen's Meeting, Bethesda, MD, December 14, 1976.
10. Clinical trials in Stage II testicular cancer. Cooperative Studies Discussion Group Meeting on Testicular Cancer, Bethesda, Maryland, December 8, 1976.
11. Current trials in the combined management of solid tumors. New York Roentgen Society Meeting, New York, NY, December 20, 1976.
12. Combined modality studies in lung, colon, and breast cancer. Albert Einstein College of Medicine, Bronx, NY, December 21, 1976.

13. Need for multimodality input in head and neck trials. Executive Council Meeting of the American Society for Head and Neck Surgeons, Bermuda, January 8, 1977.

14. General areas deserving investigation in treatment of melanoma. Cooperative Studies Discussion Group on Melanoma, Bethesda, MD, January 18, 1977.

15. Research Projects within the Cancer Therapy Evaluation Program. Orientation Week, Clinical Oncology Program, Division of Cancer Treatment, Bethesda, MD, January 25, 1977.

16. New drugs for Phase II trials. Southwest Oncology Group Meeting, Houston, TX, January 27, 1977.

17. Need for multimodality input in head and neck trials. Society of Surgical Oncology Meeting, Chicago, Illinois, January 29, 1977.

18. Review of development of Head and Neck Advisory Group and aims. Head and Neck Cancer Advisory Group Meeting, Bethesda, Maryland, February 1, 1977.

19. Review of development of Endometrial Cancer Advisory Group and aims. Endometrial Cancer Study Group Meeting, Bethesda, Maryland, February 7, 1977.

20. Review of aims of contracts for Stage I/II resectable lung cancer. Lung Contractors' Meeting, Bethesda, Maryland, February 14, 1977.

21. Opening remarks on clinical trials with vinca alkaloids. Vinca Alkaloid Meeting, Bethesda, MD, February 16, 1977.

22. Recent administrative and policy issues in the cooperative groups. Statistical Studies Discussion Group, Bethesda, Maryland, March 3, 1977.

23. General aspects of anticancer drug evaluation. International Symposium on the Chemotherapy of Oncological Diseases, London, England, March 17, 1977.

24. Chemotherapy of small cell carcinoma. The British Thoracic and Tuberculosis Association and the Norwegian Thoracic Society Meeting, Bournemouth, England, March 18, 1977.

25. Role of adjuvant chemotherapy in operable breast cancer. New York Roentgen Society Spring Conference, New York, NY, April 29, 1977.

26. Panel discussion participant on "Lung" to the Society of Head and Neck Surgeons and the Society of Surgical Oncology Meeting, Hilton Head, SC, May 7, 1977.

27. Marker studies through the Frederick Cancer Research Center. Gastrointestinal Tumor Study Group Meeting, Chicago, IL, May 11, 1977.

28. The clinical evaluation of new antitumor antibiotics. U.S. - Japan Symposium on Antitumor Antibiotics, San Francisco, CA, May 13, 1977.

29. Opening and Closing Remarks to the Second NCI Lung Cancer Conference, Airlie House, Airlie, VA, May 22-24, 1977.

30. Cis-platinum - Current status of trial and unusual adverse drug reactions: hypersensitivity and neurotoxicity. Cancer and Leukemia Group B Meeting, New York, NY, June 17, 1977.

31. New developments with anthracycline antibiotics. Johns Hopkins Hospital, Baltimore, MD, June 30, 1977.

32. Organizational Structure of the Cancer Therapy Evaluation Program. Orientation lectures, Bethesda, MD, July 5, 1977.

33. Review of studies with cytotoxic-hormone combinations. Hormone - Cytotoxic Agent Meeting, Bethesda, MD, July 15, 1977.

34. Status of current adjuvant trials. Belohorizonte, Brazil, August, 1977.

35. Review of cis-platinum: A new antitumor agent. Buenos Aires, Argentina and Lima, Peru, August, 1977.

36. Current therapeutic results in testicular cancer. Buenos Aires, Argentina and Lima, Peru, August, 1977.

37. Introduction to Symposium on New Antitumor Compounds: New Mitotic Inhibitors. 10th International Congress of Chemotherapy, Zurich, Switzerland, September, 1977.

Marcel Rozencweig, M.D.:

1. Correlation between experimental activity of anticancer agents and their hematologic toxicity in man. American Society of Hematology, Boston, MA, December, 1976.

2. Clinical comparison of vinblastine and vincristine. Vinca Alkaloids Symposium, Bethesda, MD, February 16, 1977.

3. New agents: Selected problems in drug development and clinical testing. Hahneman Symposium, Philadelphia, PA, April, 1977.

IX. Committee Participation

Franco M. Muggia, M.D.

Vice-Chairman, Combined Modality Committee
Member, Decision Network
Member, Cancer Treatment Program Staff
Member, Breast Cancer Task Force
Member, Clinical Research Subpanel
Member, Actinomycin D Analog Committee
Member, Alkylating & Nitrosourea Analog Committee
Member, Clinical Advisory Group for Viral Oncology
Member, VA Review Committee
Member, Operating Committee
Member, Drug Evaluation Subcommittee
NCI Coordinator for Chemotherapy, U.S. - Japan Agreement
Chairman, Lung Cancer Program, U.S. - U.S.S.R. Agreement

X. Conferences sponsored by the CTEP

1. Lung Cancer Advisory Group, July 29, 1977, Bethesda
2. Head and Neck Cancer Advisory Group, August 5, 1977, Bethesda
3. Osteosarcoma Study Group, January 19, 1977, Bethesda
4. Head and Neck Cancer Advisory Group, February 1, 1977, Bethesda
5. Endometrial Cancer Study Group, February 7, 1977, Bethesda
6. New Drug Liaison Meeting, February 17-18, 1977, Bethesda
7. Phase I Working Group, April 26-27, 1977, Bethesda
8. Second NCI Lung Cancer Conference, May 22-24, 1977, Airlie House, Airlie, Virginia
9. Endometrial Cancer Study Group Meeting, July 11, 1977, Bethesda
10. Hormone - Cytotoxic Agent Meeting, July 15, 1977, Bethesda
11. Cooperative Discussion Groups:
    a. Non-Hodgkin's Lymphoma, May 18, 1976, Bethesda
    b. Adult Acute Leukemia, May 26, 1976, Bethesda
    c. Pediatric Solid Tumors, June 26, 1976, Bethesda
    d. Bladder Cancer, October 26, 1976, Bethesda
    e. Testicular Cancer, December 8, 1976, Bethesda
    f. Malignant Melanoma, January 18, 1977, Bethesda
    g. Statistics, March 3, 1977, Bethesda
    h. Adjuvant Therapy of Colon Cancer, March 8, 1977, Bethesda

XI. Foreign Visitors

1. Dr. Umezawa - Tokyo, Japan - July 9, 1976
2. Dr. Maier - Germany - July 27, 1976
3. Dr. Ilbery - Australia - July 30, 1976
4. Dr. Cugusi - Milan, Italy - September 10, 1976
5. Dr. Olivari - Buenos Aires, Argentina - October 28, 1976
6. Dr. Gisselbrecht - Paris, France - November 17, 1976
7. Dr. Horikoshi - Tokyo, Japan - November 17, 1976
8. Dr. Espana - Madrid, Spain - November 17, 1976

9. Dr. Heuson - Brussels, Belgium - November 17, 1976
10. Dr. Mourali - Tunis, Tunisia - November 26, 1976
11. Dr. Galmarini - Buenos Aires, Argentina - January 25, 1977
12. Dr. Prabier - Buenos Aires, Argentina - January 31, 1977
13. Dr. Muldonado - Caracas, Venezuela - April 18, 1977
14. Dr. Markovic - Yugoslavia - April 20, 1977
15. Dr. Salem - Beirut, Lebanon - April 12, 1977
16. Dr. Lilieveld - Rijswijk, The Netherlands - May 25, 1977
17. Dr. Bringel - Stockholm, Sweden - May 25, 1977
18. Dr. Sealy - Capetown, South Africa - May 26, 1977
19. Dr. Britton - Newcastle, England - June 9, 1977
20. The Drs. Falkson - Pretoria, South Africa - June 13, 1977
21. Dr. Van Bekkum - Rijswijk, The Netherlands - June 21, 1977

APPENDIX

AGENDA

SECOND NCI LUNG CANCER CONFERENCE

Sunday, May 22

1:00 - 6:00 p.m.	Registration
6:00 - 7:30 p.m.	Reception
7:30 p.m.	Dinner
	Comments and Introduction of Foreign Guests - Oleg S. Selawry

Monday, May 23

8:00 a.m.	INTRODUCTORY SESSION
	Welcoming Remarks - Franco M. Muggia
	Introduction to the Second NCI Conference on Lung Cancer Treatment - Stephen K. Carter
8:30 - 10:30 a.m.	SIMULTANEOUS WORKSHOPS

SURGERY IN STAGING AND TREATMENT
Chairman: Clifton F. Mountain

Management Implications Derived from Surgical Staging Studies - C.F. Mountain and D.L. Paulson

The Identification and Treatment of Clinically Occult Cancer of the Lung - R.R. Baker and W.S. Payne

The Identification and Prognostic Implications of Positive Mediastinal Lymph Nodes - N. Martini and M.M. Kirsh

An Assessment of Tumor Markers in the Management of the Surgical Patient - R.G. Vincent and J.P. Concannon

New Methods in the Surgery of Lung Cancer - A.P. Naef and E.W. Wilkins, Jr.

Selected Abstracts

ROLE OF RADIOTHERAPY
Chairman: Carlos A. Perez

Radiotherapy in the Treatment of Lung Cancer: An Overview - C.A. Perez

Time-Dose Relationship in Tumor Control - O.M. Salazar

Preoperative Radiation - H.C. Kent

Patterns of Failure Following Treatment of Localized Bronchial Carcinoma - J.D. Cox

Combined Radiotherapy and Chemotherapy in Non-Small Cell Lung Cancer - R. Sealy

Combined Radiotherapy and Chemotherapy in Small Cell Carcinoma - N.M. Bleehen

Selected Abstracts

EXPERIMENTAL CHEMOTHERAPY AND TUMOR CELL KINETICS
Chairman: Frank M. Schabel, Jr.

Overview of Experimental Tumor Systems - F.M. Schabel, Jr.

The Use of Human Tumor Xenografts for Selection of New Agents Against Tumors with Poor Response to Established Drugs - R.K. Johnson

Combined Modalities in Experimental Systems - L.M. van Putten

Combined Modalities: Implications Derived from Cell Kinetics - M. Tubiana

Application of Principles of Cell Kinetics in the Treatment of Small Cell Carcinoma of the Lung - S.E. Shackney

Selected Abstracts

10:30 - 11:00 a.m. COFFEE BREAK

Monday, May 23

11:00 - 1:00 p.m. SIMULTANEOUS WORKSHOPS

PATHOLOGY AND DIAGNOSIS OF LUNG CANCER

Chairmen: Raymond Yesner, Mary J. Matthews, and Leslie H. Sobin

Overview of Problems of Diagnosis - R. Yesner

Early Diagnosis of Lung Cancer - D. Carter

Surgical Pathology of Resectable Lung Cancer - F. Rilke

Cyto-Histopathologic Correlations in Lung Cancer - J.M. Lukeman

Cell-Type Specificity in Pulmonary Pathology - L.B. Woolner

Bronchial Epithelial Changes in Association with Small Cell Carcinoma of the Lung - A.F. Gazdar

Epidemiology and Pathology of Mesothelioma in South Africa - C.J. Uys

Effects of Therapy on the Morphology and Behavior of Small Cell Carcinoma of the Lung - M.J. Matthews

The WHO Histological Classification of Lung Tumors - L.H. Sobin

Selected Abstracts

ROLE OF CHEMOTHERAPY FOLLOWING SURGERY

Chairman: Gianni Bonadonna

Adjuvant Chemotherapy in Lung Cancer: An Overview - S.S. Legha

Studies with Cyclophosphamide - K.W. Brunner

Effectiveness of Oral CCNU as an Adjuvant to Curative Surgery - C.F. Mountain and B. Peterson

Experience of the VA Surgical Adjuvant Group - G.A. Higgins

Controlled EORTC Study with Adjuvant Radiotherapy, Chemotherapy and Immunotherapy in Operable Epidermoid Bronchial Carcinoma - G. Bonadonna

Prospects and Design of Future Trials - G. Bonadonna

Selected Abstracts

NEW THERAPEUTIC APPROACHES
Chairman: Philip Rubin

Future Directions in the Treatment of Lung Cancer - P. Rubin

Systemic Irradiation by the Half-Body Technique - W.D. Rider

Role of Radiosensitizers - T.L. Phillips and D. Chapman

Role of Hyperthermia - R. Johnson and M.L.M. Boone

Selected Abstracts

1:15 p.m. LUNCH

2:15 - 4:15 p.m. SIMULTANEOUS WORKSHOPS

SYSTEMIC THERAPY OF NON-SMALL CELL CARCINOMA
Chairmen: Pierre Alberto and Martin H. Cohen

General Accomplishments of Systemic Therapy - J. Wolf

Treatment with Single Agents - N.I. Perevodchikova and M.H. Cohen

Combination Chemotherapy in Non-Small Cell Lung Cancer - R.T. Eagan

Statistical Considerations in the Planning and Analysis of Non-Small Cell Lung Cancer Studies - M. Zelen

The Design of Clinical Studies in Advanced Lung Cancer - P. Alberto

Selected Abstracts

TUMOR IMMUNOLOGY
Chairman: Paul B. Chretien

General Concepts and Immune Reconstitution - P.B. Chretien

Identification of Antigens Associated with Human Lung Cancer Suitable for Diagnosis and Monitoring - K.R. McIntire

Evaluation of Host Factors - E.C. Holmes

Immunotherapy of Lung Cancer with Oil-Attached Cell-Wall Skeletons of M. bovis BCG and N. rubra - Y. Yamamura

Immune Stimulation with Cell Fractions and Vaccine - A.C. Hollinshead

Relevance of Immunologic Analysis in Patients with Inoperable Lung Cancer Treated by Radiotherapy - S. Stefani

Selected Abstracts

4:30 - 6:30 p.m. SIMULTANEOUS WORKSHOPS

ROLE OF IMMUNOTHERAPY FOLLOWING SURGERY

Chairman: Roy E. Ritts, Jr.

Rationale for Immunoprophylaxis in Resectable Early Lung Cancer - R.E. Ritts, Jr.

Regional Immunotherapy Using Intrapleural BCG and Isoniazid - M.F. McKneally

Immunotherapy of Stage I and II Squamous Cell Lung Cancer - G. Mathe

Studies with Other Immunoadjuvants - E.C. Holmes

Prospects for Specific Immunotherapy - R.B. Herberman

Selected Abstracts

STAGING AND FOLLOW-UP STUDIES IN ADVANCED LUNG CANCER

Chairman: Heine H. Hansen

Methods of Evaluation; Skeletal and Hepatic Metastases - H.H. Hansen

Role of Fiberoptic Bronchoscopy - D.C. Ihde

Tumor Markers: Placental Proteins - S.W. Rosen and L.E. Broder

Monday, May 23

Ectopic ACTH in Bronchogenic Carcinoma - R.S. Yalow

Ectopic Hormone Production in Small Cell Carcinoma as Related to Stage and Subclassification - M. Hansen

Selected Abstracts

7:00 p.m. DINNER

8:30 - 10:30 p.m. JOINT WORKSHOP

PROSPECTS FOR CURE OF SMALL CELL CARCINOMA
Chairmen: Yvon Kenis and John D. Minna

Salient Problems in the Treatment of Small Cell Carcinoma - Y. Kenis

Review of Current Trials: Approach to CNS Metastases - P.A. Bunn and R.B. Livingston

Chemotherapy and Radiotherapy for Small Cell Lung Cancer: A Remedy for Past Therapeutic Failure - H.D. Brereton

Feasibility and Results of Intensive Chemotherapy - M.H. Cohen

Summary and Therapeutic Alternatives - J.D. Minna

Selected Abstracts

Tuesday, May 24 PLENARY SESSION

8:00 - 9:15 a.m. Summary of Workshops on Early Lung Cancer (Workshop Chairmen)

9:30 - 10:45 a.m. Summary of Workshops on Advanced Lung Cancer (Workshop Chairmen)

11:00 - 12:00 noon Summary of Workshops on New Approaches (Workshop Chairmen)

12:00 - 12:30 p.m. Concluding Remarks - Franco M. Muggia

12:30 p.m. LUNCH

CLINICAL INVESTIGATIONS BRANCH

Introduction

The Clinical Investigations Branch (CIB) is responsible for the scientific administration of the Cooperative Group Program. The Chief of the CIB is directly responsible to the Associate Director, Cancer Therapy Evaluation Program (CTEP).

The clinical cooperative groups were first organized in 1956 to study clinical drug development in leukemia. During the 1960's the groups expanded to test new agents in solid tumors. They provided the majority of the information on activity of new drugs through Phase I and II testing. As interest in combined modality approaches to cancer treatment grew, the image of the groups changed to reflect this new approach. In August of 1975 the cooperative group program was incorporated into the Division of Cancer Treatment in the interest of better coordination and centralization of therapeutic research. The CIB, thus, is a branch of the CTEP, one of four large programs of the DCT.

As the complexity of clinical trials evolved, the groups exhibited a period of controlled growth. In FY'77 there were a total of 189 grants in 16 groups funded for $26,627,000.

The groups cover a wide range of activities and vary from large multidiscipline, multidisease study groups to single activity groups.

I. Multimodal Multidisease Groups

1. Cancer and Leukemia Group B (CALGB)
2. Eastern Cooperative Oncology Group (ECOG)
3. Southeastern Cancer Study Group (SEG)
4. Southwest Oncology Group (SWOG)

II. Multimodality Groups Devoted to a Major Oncologic Area

1. Children's Cancer Study Group (CCSG)
2. Gynecologic Oncology Group (GOG)

III. Single Modality Group

1. Radiation Therapy Oncology Group

IV. Single Disease Groups

1. Primary Breast Cancer Therapy Group (PBTG-NSABP)
2. National Wilms' Tumor Study Group (NWTG)
3. Polycythemia Vera Group (PVG)
4. Malignant Melanoma Group (MMG)
5. Uro-Oncology Research Group (UROG)

V. Special Activities Groups

1. Lymphoma Pathology Reference Center (LPRC)
2. Radiologic Physics Center (RPC)
3. Clinical Coordinating Center (CCC)
4. European Organization for Research on Treatment for Cancer (EORTC)-Operations and Statistical Office

In addition, the CIB has a functional, not administrative, relationship with two major Veterans Administration study groups, and a third DCT contract supported group.

1. The Veterans Administration Lung Cancer Group (VALG)
2. The Veterans Administration Surgical Adjuvant Group (VASAG)
3. The Brain Tumor Study Group (BTSG)

Basically, the Type I groups are large, and a major highlight of FY'76-'77 was their expansion to a full range of multidisciplinary activities. An illustration of this is the transformation of the Acute Leukemia Group B to the Cancer and Leukemia Group B. This group had long enjoyed an international reputation for its studies of acute luekemia. In 1973 the group began to expand into breast cancer and osteosarcoma. At the present time there are active committees for surgery, immunology, pathology, radiation therapy, and psychiatry, with protocols in operation or in the planning stage.

The Eastern Cooperative Oncology Group grew out of the old Eastern Solid Tumor Study Group. It remains heavily committed to solid tumor studies, but has substantially increased its activities in combined modality studies and in hematologic malignancies.

The Southeastern Group underwent a reorganization of major proportions in the last two years. Before that, careful studies in leukemia and lymphoma were its trademark. They have now undertaken successful ventures of combined modality treatment in lung cancer and melanoma, and exhibit great potential for multidisciplinary studies in head and neck cancer.

The Southwest Oncology Group was a real leader in multidisciplinary organization. Largest of the groups, with an annual case accrual of 6,000 patients, this group possesses the size and flexibility to rapidly carry out Phase III and IV studies.

The Type II groups have been multidisciplinary from their inception. Surgical and radiotherapy input has been major in the Children's Group studies for several years. The Gynecologic Oncology group likewise has developed an equal partnership of gynecologists, radiotherapists, and medical oncologists, and the majority of their protocols involve the three disciplines.

The Radiation Therapy Oncology Group is based on the application of a single therapeutic modality; however, they have explored the combination of radiation therapy with chemotherapy. New directions include high LET radiotherapy techniques and the use of hyperthermia and radiosensitizers.

The Type IV single disease groups have provided major insights into their chosen areas. The early successes of adjuvant chemotherapy in operable breast cancer are credited to the PBTG (NSABP), and the multidisciplinary treatment of Wilms' tumor is the major accomplishment of the NWTG.

The special activities groups provide unusual services for their clients.

The Lymphoma Pathology Reference Center provides expert review of pathological material for the groups performing therapeutic research in malignant lymphoma.

Quality control and accurate dosimetry monitoring are vital in radiation therapy. The RPC provides the initial calibration of all radiotherapy equipment and aids the groups in the necessary continuing quality control.

The Clinical Coordinating Center is a large statistical center located at SUNYAB which provides data processing and statistical services and consultation for ECOG and RTOG, as well as contract supported organizations, the VALG and GITSG. This provides a critical mass of statistical talent. This approach has led to some noteworthy contributions to statistical science in clinical trials.

The operations and statistical office of the EORTC is analagous to the CCC. This office is the "glue" binding the multiple cooperative groups, working parties, and committees that comprise the EORTC.

In summary, the cooperative clinical groups are diverse and encompass the entire scope of cancer treatment. They comprise the entire grant supported extramural program of the DCT, and complement the extensive intramural and contract programs of that division.

Organization of the CIB

The CIB occupies offices in close proximity to the review committee for the groups, the Cancer Clinical Investigation Review Committee (CCIRC).

Personnel

1. Hugh L. Davis, Jr., M.D. - Special Assistant for the CIB, CTEP, DCT
2. Edwin M. Jacobs, M.D. - Program Director, CIB, CTEP, DCT
3. Mrs. Barbara Shepherd - Secretary to the Special Assistant, CIB
4. Ms. Elaine Lewis - Secretary to the Program Director, CIB

Dr. Davis is responsible for the overall administration of the Branch, and coordination of its activities with the Cancer Therapy Evaluation Program, the Grants Administration Branch, the Cancer Clinical Investigation Review Committee, and the National Cancer Advisory Board.

Dr. Jacobs serves as Program Director. He is responsible for ongoing program review, the quality control of the groups, and shares overall administrative responsibilities with Dr. Davis. The office management is ably conducted by Mrs. Shepherd, and the large volume of material of the ongoing review, by Ms. Lewis.

Quality control is an integral part of the activities of this branch and is outlined below.

1. Protocol Review:

   Six copies of the final draft of a group protocol are submitted to the Program Director, CIB, for review. The protocols are reviewed by the CIB staff, consultants from appropriate areas of the NCI, and when applicable, consultants from outside the NCI. Two or more reviewers furnish the Program Director, CIB, with a written review of the scientific merit, uniqueness, cost-benefit ratio, and ethics of the study. The Program Director, CIB, then transmits any concerns to the group chairman and negotiates revisions, if necessary.

   A protocol which is approved then is sent to the Investigational Drug Branch for filing with the FDA.

2. Requirements for Reporting:

   a. The CIB requires full reporting of all group studies in the minutes of the groups. When the study is terminated a final report is required in the minutes. This includes:

      1) An introduction.
      2) A protocol schema.
      3) Results:

         a) Number of patients entered, eligible, evaluable.
         b) Toxicity analysis.
         c) Results of the therapeutic regimens.
         d) An appropriate statistical analysis.

   b. The groups send 44 copies of the minutes of each meeting to the CIB which is responsible for distribution to the CTEP and the Review Committee members (CCIRC).

   c. The CIB requires an annual progress report from each ACTIVE group.

3. Attendance at Group Meetings:

   A member of the CIB staff attends each meeting of the cooperative groups and assesses the overall performance of the group and sends a written report to the Associate Director, CTEP

4. Sponsoring Cooperative Discussion Groups:

   Disease-oriented cooperative discussion groups provide a mechanism for assessment of overall performance. These are held at appropriate intervals and are sponsored by the CIB.

5. Interactions with the Group Chairmen:

The CIB organizes regular meetings of the cooperative group chairmen and the DCT staff.

6. CIB Sponsored Program Reviews:

When appropriate, the staff of the CIB conducts a formal program review of a cooperative group.

The CIB staff may select consultants from the NCI and elsewhere to assist in the performance of this review. The CIB staff and consultants meet with the group chairman and key group investigators for an in depth discussion and review of the operation of the group. A written report is generated and recommendations are transmitted to the group chairman and the Associate Director, CTEP.

7. Coordinating Functions:

The CIB also acts as a "coordinating center" for the interaction of the cooperative groups with the Grants Administration Branch, the Office for Protection from Research Risks (OPRR), and other NCI activities.

A Chronology of the CIB Activities Since the Last Report

1. Cooperative Discussion Groups

   a. Non-Hodgkin's Lymphoma, May 18, 1976, NIH, Bethesda
   b. Adult Acute Leukemia, May 26, 1976, NIH, Bethesda
   c. Pediatric Solid Tumors, June 26, 1976, NIH, Bethesda
   d. Bladder Cancer, October 26, 1976, NIH, Bethesda
   e. Testicular Cancer, December 8, 1976, NIH, Bethesda
   f. Malignant Melanoma, January 18, 1977, NIH, Bethesda
   g. Statistics, March 3, 1977, NIH, Bethesda
   h. Adjuvant Therapy of Colon Cancer, March 8, 1977, NIH, Bethesda

The discussion groups have served to identify gap areas in the program. They have also fostered interchange of scientific ideas and identified areas for profitable intergroup participation. Particularly noteworthy in this regard were the discussion groups in melanoma, colon, and testicular cancer. An intergroup effort is well under way as a result of the testicular conference. The interaction of the group statisticians was long overdue and the statisticians plan to hold regular meetings for information exchange.

Future meetings planned for fall '77 - spring '78

1. Sarcomas
2. Pediatric Intergroup Studies
3. Statistics
4. Breast Cancer (Advanced and Early)

Program Reviews and Interpretation of Merit Review Documents to Group Investigators

1. Program Review of the GOG, August 18, 1976.

   This was accomplished by an on-site visit to the GOG headquarters by CIB staff and consultants from the CMB and the MB, COP, DCT. This review served to identify areas of concern in the GOG and mechanisms for correction of deficiencies were worked out and promptly implemented by the group.

2. Post Review Conference with the Chairman and Executive Committee of the CCSG, October 6, 1976.

   This was the first of an ongoing effort to provide detailed information on the merit review of each group conducted by the CCIRC.

3. Post Review Conference with the Chairman of the PBTG, October 12, 1976.

4. Post Review Conference with the Chairman of the CALGB, February 22, 1977, and with the Principal Investigators of the CALGB, March 18, 1977.

The CIB proposes to continue this program with a visit to the group headquarters shortly after the Board Review of each renewal grant. It is felt that ongoing exploration of the results of the review process will make the groups more responsive to review, and result in better overall group performance, as well as mutual understanding and trust.

Regular Conferences with the Group Chairmen

The CIB is responsible for the organization of regular conferences between the cooperative group chairmen, the Director, DCT, and the Associate Director, CTEP, DCT. The entire body of the group chairmen meets twice yearly, and meetings of the smaller executive committee of the group chairmen are generally held quarterly. Policy matters of mutual concern are discussed.

These conferences are particularly useful to thoroughly explain DCT policy and implement policy of the NIH, OPRR, and FDA. The CIB is responsible for the day to day implementation of this policy, and this personal contact with the group chairmen is thus, particularly useful.

Other Functions

The CIB also participates in other activites of the CTEP. Dr. Davis is acting project officer for the Wayne State University Phase II/III contract, and Dr. Jacobs is project officer for the Memorial-Sloan Kettering Phase II/III contract.

Presentations

1. "Investigational Drugs in Lung Cancer" - Hugh L. Davis, Jr., M.D. Presented at the VALG meeting, May 27, 1976, New Orleans, La.

2. "Protocol Review by the CIB" - E.M. Jacobs, M.D. Presented at the RTOG meeting, June 15, 1976, Hyannis, Mass.

3. "An Overview of Investigational Drugs" - Hugh L. Davis, Jr., M.D. Presented at the SWOG meeting, October 21, 1976, Troy, Michigan.

4. "The Process of Drug Development" - Hugh L. Davis, Jr., M.D. Presented at the annual meeting of the Nursing Oncology Association, November 8, 1976, Boston, Mass.

5. "Adjuvant Trials in Breast Cancer" - Hugh L. Davis, Jr., M.D. Presented at the University of Virginia, March 29, 1977, Charlottesville, Va.

6. "The Role of Antitumor Antibiotics in Oncologic Practice" - Hugh L. Davis, Jr., M.D. Presented at the US-Japan Symposium on Antitumor Antibiotics, May 12-13, San Francisco, California.

7. "Current Adjuvant Trials in Breast Cancer" - Hugh L. Davis, Jr., M.D. Presented at the US-USSR Joint Conference on Breast Cancer, May 24-25, 1977, Bethesda, Md.

8. "Evaluation and Role of Single Chemotherapeutic Agents in Adult Non-Hodgkin's Lymphoma" - Hugh L. Davis, Jr., M.D. Presented at the CCNRC Symposium on Lymphoid Neoplasia, June 23-25, 1977, Paris, France.

COMBINED MODALITY BRANCH (CMB)

This Branch is responsible for developing disease-oriented strategies and for monitoring and selecting contract-supported activities which emanate as a result of this strategy. A major goal during the past year has been to integrate the grant-supported activities in the cooperative groups - which are monitored by the Clinical Investigations Branch (CIB) - within these strategies. Ultimately, a fusion of the two Branches has been proposed to optimally coordinate the two programs (Figure 1).

Figure 1 Clinical Investigations Branch

1. Office of the Chief
    Program Director - Cooperative Groups
    Assistant Program Director - Same

2. Medical Therapy Evaluation Section
    Modality & Disease Coordinators

3. Radiotherapy Evaluation Section
    Modality & Disease Coordinators

4. Surgical Therapy Evaluation Section
    Modality & Disease Coordinators

Dr. William McGuire joined the staff as Senior Investigator, assuming many responsibilities within the Program. Because of the wide scope of activities, the CMB often also recruited the assistance of the CIB staff as disease coordinators and project officers throughout FY 1977. With the departure of Dr. Harry Handelsman, additional personnel, including others with the ability to fully represent additional therapeutic modalities in the Program, are expected to join the newly created combined Branch in July, 1977.

The current report deals specifically with:

1) Description and highlights of Phase II and III contract-supported studies in various solid tumors.

2) Description and highlights of disease specific contract-supported studies.

3) New developments in the contract program as a result of evolving strategies.

4) Activities of the peer-review committees monitoring the contracts.

I. Phase II and III Studies in Disseminated Solid Tumors

These contracts were conceived to serve a dual role: 1) to quickly test new agents in order to obtain Phase II data on the signal tumors and 2) to develop pilot combination programs in various tumors as new leads arise, with emphasis in the common malignancies.

Table 1 indicates the five contracts under this category and the various project officers. Table 2 indicates the wide scope of programs under each contract. Increasing input from staff and coordination is expected to increase the yield of pace-setting studies. In addition, one may conceive of these contracts as providing the basic ingredients for contract-supported activities which are evolving in various disease areas (see Section II and III).

Among the highlights from these contracts during the past year are:

1) Establishing the effectiveness of a combination including cis-platinum in testicular cancer (VAB III; Memorial Hospital).

2) Introducing cis-platinum as a promising agent in head and neck cancer and in bladder cancer (Memorial Hospital).

3) Identifying clear activity of combination chemotherapy relative to single agents in non-small cell lung cancer, associated with prolongation in survival (cytoxan-Adriamycin-cis-platinum, Mayo Clinic).

4) Introducing VP-16 in first and second line combination treatments in small cell lung cancer (Mayo Clinic).

5) Providing the basis for a randomized study in osteosarcoma for delayed versus immediate adjuvant chemotherapy (Mayo Clinic).

6) Identifying therapeutic synergism between actinomycin D and DTIC in the treatment of advanced malignant, predominantly visceral, melanoma (M.D. Anderson and Wayne State).

7) Determining the Phase II activity of high dose methotrexate in a variety of solid tumors, with promising results in head and neck cancer, breast cancer and malignant lymphoma (Sidney Farber).

In addition, vast experience was added to the available data on many investigational drugs such as ifosfamide, ftorafur, epipodophyllotoxins, pyrazofurin, ICRF, Baker's (triazenate), dianhydrogalactitol, piperazinedione, etc....

TABLE 1: PHASE II/III STUDIES IN DISSEMINATED SOLID TUMORS

CONTRACT NUMBER	TITLE	PROJECT OFFICER	CONTRACTORS	PRINCIPAL INVESTIGATOR
57044	Phase II/III Studies in Patients with Disseminated Solid Tumors	Handelsman	Mayo Foundation	Dr. John Edmonson
57043	Phase II/III Studies in Patients with Disseminated Solid Tumors	Jacobs	Memorial Hospital	Dr. Joseph Burchenal
57042	Phase II/III Studies in Patients with Disseminated Solid Tumors	Kisner	M.D. Anderson	Dr. Gerald Bodey
67037	Phase II/III Studies in Patients with Disseminated Solid Tumors	McGuire	Sidney Farber Cancer Center	Dr. George Canellos
67105	Phase II/III Studies in Patients with Disseminated Solid Tumors	Davis	Wayne State University	Dr. Laurence Baker

TABLE 2: PHASE II/III CONTRACTS: SIGNAL TUMORS

MAYO

LUNG
OVARIAN
CERVICAL
ENDOMETRIAL
HEAD AND NECK
PROSTATE
BLADDER
MELANOMA
SOFT TISSUE SARCOMA

M.D. ANDERSON

LUNG
BREAST
MELANOMA
SOFT TISSUE SARCOMA
BONE SARCOMA

MEMORIAL

LARGE BOWEL
LUNG
BREAST
GASTRIC
PROSTATE
BLADDER
RENAL
OVARIAN
CERVICAL
ENDOMETRIAL
MELANOMA
PANCREAS

SIDNEY FARBER

LARGE BOWEL
LUNG
LYMPHOMA
HEAD AND NECK
ENDOMETRIAL
OVARIAN
BLADDER
SOFT TISSUE SARCOMA
BONE SARCOMA
PROSTATE
BREAST

WAYNE STATE

LARGE BOWEL
LUNG
LYMPHOMA
HEAD AND NECK
ENDOMETRIAL
OVARIAN
BLADDER
SOFT TISSUE SARCOMA
BREAST
PROSTATE
BONE SARCOMA

Future directions of these contracts will be covered in Section III.

II. Disease-Specific Contract-Supported Studies

A. Lung Cancer. Studies being carried out by the Working Party for Therapy of Lung Cancer (WPL) were terminated December 31, 1976, as the contracts were recompeted. A new group has been formed to evaluate adjuvant treatments in resectable patients (see Section III).

The WPL was designed in 1972 to study single agents, drug combinations, and combined modality therapy of lung cancer. The first two years were totally directed at studies in advanced disease and conclusions were that neither combinations of drugs nor combined modality therapy made any significant impact on survival in this group of patients. The effort was therefore redirected toward earlier, resectable disease, and the contracts were terminated, with a recompetition towards this new goal initiated.

The Veterans Administration Lung Group has continued to do studies in unresectable disease, both limited and extensive. Recent studies have demonstrated that the combination of adriamycin and cytoxan is superior to cytoxan alone in all cell types of lung cancer.

B. GI Cancer. Several contracts are concerned specifically with gastrointestinal cancer. Most of these fall under the structure developed to carry out joint adjuvant studies in colon cancer and additional joint studies, including advanced disease, in gastric and pancreatic cancer (GITSG).

Gastrointestinal Tumor Study Group (GITSG) - This group counts with a contract-supported statistical office (statisticians, Philip Lavin and John MacIntyre) and holds twice-yearly, joint meetings under the chairmanship of Drs. Charles Moertel and Philip Schein. Table 3 indicates the type of studies and the patient accrual as of the January, 1977 meeting.

Important landmarks of the GITSG studies during FY 1977 include:

1) Demonstration of ineffectiveness of 6000 rads in treatment of locally unresectable pancreatic cancer.

2) Demonstration of the superiority of 5FU alone over radiation plus 5FU in locally unresectable gastric carcinoma.

3) Establishing the prognostic value of preoperative CEA in resectable colorectal cancer.

Most of the conclusions from the adjuvant studies are not yet in requiring additional years of patient accrual and evaluation.

TABLE 3. Patient Accession by GITSG Protocol and Institution (11/19/76)

	COLON			GASTRIC				PANCREAS				
	6175	7175	7276	8174	8274	8375	8376	9173	9273	9374	9376	Total
	COLON ADJUVANT	RECTUM ADJUVANT	LOCALLY UNRESECTABLE RECTUM	ADJUVANT	LOCALLY UNRESECTABLE	ADVANCED (CLOSED)	NEW ADVANCED	RESECTABLE	OLD LOCALLY UNRESECTABLE	ADVANCED (CLOSED)	NEW ADVANCED	
Mayo Clinic	30	13	0	22	9	54	16	6	56	32	9	247 (25%)
Sidney Farber	31	6	0	9	16	21	0	3	14	4	0	104 (11%)
Roswell Park	22	13	2	17	16	23	6	5	12	10	6	132 (13%)
UCLA	12	1	0	3	6	0	0	3	21	1	0	47 (5%)
Yale	4	2	0	10	16	20	3	0	22	11	6	94 (10%)
USC	12	5	0	8	15	15	3	2	15	7	1	83 (8%)
Miami	5	2	-	-	-	-	-	6	21	15	20	69 (7%)
Mt. Sinai-NY	11	2	2	19	17	41	12	-	-	-	-	104 (11%)
Albany	26	10	0	-	-	-	-	-	-	-	-	36 (4%)
Memorial	9	9	0	-	-	-	-	-	-	-	-	18 (2%)
Milan	5	10	0	-	-	-	-	-	-	-	-	15 (2%)
Georgetown	15	3	5	-	-	-	-	-	-	-	-	23 (2%)
Chicago	5	3	0	-	-	-	-	-	-	-	-	8 (1%)
Total	187	79	9	88	95	174	40	25	161	80	42	980

Additional studies in gastrointestinal cancer are being carried out by the Veterans Administration Surgical Adjuvant Group (testing the value of chemotherapy following resectable colon and gastric cancer, preoperative radiotherapy in rectal cancer, preoperative radiotherapy in esophageal cancer is also forthcoming). Phase III studies in colon and gastric cancer represent also an effort by the University of Leeds (England) that includes evaluation of markers in this small and targeted program effort.

At Georgetown University, developmental work investigating nutritional consequences of treatment of GI cancer and pilot treatment programs in all aspects of GI neoplasia are being launched under a contract separate from GITSG. Highlights of this contract to date have been: 1) Piloting a promising combination chemotherapy in gastric cancer (Fluorouracil, adriamycin, mitomycin C), 2) Clinical testing of a new nitrosourea, chlorozotocin, because of possible marrow sparing effect for future use in combination programs in GI cancer, 3) investigating important structure-activity relations of this group of compounds, and 4) The launching of various diagnostic and therapeutic studies dealing with pancreatic cancer.

Additional nutritional effects on treatment of cancer are being studied by M.D. Anderson Hospital as part of the Phase II/III contracts. These contracts are also exploring many additional regimens including new drugs in colon cancer which are urgently needed. Of course, the major effort in this direction has been provided by Dr. Charles Moertel as principal investigator of a Mayo Contract to devise and evaluate new therapies for colorectal carcinoma. Table 4 indicates the number of agents that have been evaluated since the inception of this contract.

C. Breast Cancer. In breast cancer, two contracts (Roswell Park - Dr. Dao and Mayo Clinic - Dr. Ahmann) were awarded to evaluate new Phase II studies and to develop more effective combination chemotherapy in advanced disease regimens including, in one instance, an ablative procedure such as adrenalectomy. These studies will be followed by increasing efforts in integrating endocrine with non-hormonal chemotherapy, maximally utilizing the joint expertise available by the contractors.

Highlights include: 1) Studies evaluating fixed crossover, such as between CFP and adriamycin-L Pam versus each regimen given to progression which is accruing satisfactorily and 2) Comparison of chemotherapy alone versus chemotherapy added to adrenalectomy.

Another major effort in breast cancer has been through the contract with Istituto Nazionale per lo Studio e la Cura dei Tumori (which also includes studies in brain and GI tumors). This contract has dealt primarily with adjuvant chemotherapy, and its highlights have received widespread attention:

TABLE 4: CHEMOTHERAPY OF COLORECTAL CARCINOMA

AGENT	PATIENTS	OBJECTIVE RESPONSE RATE AT 2 MONTHS (%)
SINGLE AGENTS OF NO SIGNIFICANT VALUE		
Vincristine	9	0
Bleomycin	15	0
Imidazole carboxamide	17	0
Benzcarbimine	20	0
Ifosphamide	21	0
TIC mustard	22	0
Hydroxyurea	22	0
Cytembena	25	0
5-Azacytidine	27	0
Chromomycin A_3	27	0
Streptonigrin	27	0
VP-16	28	0
Galactitol	30	0
CAC platinum	33	3
Camptothecin	49	4
Adriamycin	56	5
Methotrexate, oral	38	5
Emetine	18	6
Fluorometholone	18	6
FUDR, 24 hr. infusion	64	6
Streptozotocin	18	6
Cyclophosphamide	25	8
SINGLE AGENTS WITH SOME DEGREE OF THERAPEUTIC ACTIVITY		
5-Fluorouracil	359	17
FUDR, rapid i.v.	149	22
Mitomycin C	69	12
CCNU	75	9
BCNU	69	10
Methyl CCNU	38	18
ICRF 159	25	12

1) Establishing prolonged disease-free survival in Stage II breast cancer treated with CMF.

2) Evaluating the relationship of this effect to treatment duration (6 versus 12 months), age, menopausal status, and number of lymph nodes.

3) Designing the initial randomized study evaluating chemotherapy in Stage III breast cancer (prior and subsequent to local treatment).

4) Introducing the adriamycin-vincristine combination and testing its effectiveness as fixed crossover with CMF in advanced breast cancer.

D. Ovarian Cancer. Until 18 months ago, all efforts in ovarian cancer sponsored by the DCT Contract Program were in advanced (FIGO Stage III/IV) disease. Both M.D. Anderson Hospital and Mt. Sinai Hospital in Houston and New York, respectively, have ongoing studies which have identified and/or verified the activity of hexamethylmelamine and cis-platinum as active agents in this tumor.

Due to significant activity of several agents in this disease and the poor prognosis of most patients with earlier stages (FIGO Stage I/II) of ovarian cancer, new contracts were competed in November, 1975, to study drug and/or radiation therapy. As a result of this competition, the Ovarian Cancer Study Group was formed consisting of Mayo Clinic, M.D. Anderson Hospital, Roswell Park Memorial Institute, and the Medicine Branch, NCI. These four institutions are jointly accruing patients to two protocols which require vigorous surgical staging and addition of melphalan and/or radioisotopes intraperitoneally as adjuvants to surgery. Each institution is performing their own pilot studies in more advanced disease with the possibility of incorporating any promising leads found here into groupwide studies.

E. Melanoma. We have a group sponsored by the World Health Organization (WHO) doing clinical trials in this disease. WHO Clinical Trial #6 is the study of the value of adjuvant DTIC, BCG, DTIC + BCG or no further therapy following lymph node dissection in patients with nodal metastases or Stage L lesions of the trunk. As of March 25, 1977, 300 cases have been randomized on this study. The results are still preliminary, but actual analysis reveals that there is a statistically significant difference between each of the treatment arms compared to the control surgical group at 12 months. Specific differences between each of the treatment arms themselves are not significant at this point. Obviously longer followup is needed. Clinical Trial #8 now has 45 cases enrolled in the last 8 months. The study is assessing the value of chemotherapy or chemotherapy plus immunotherapy in advanced metastatic malignant melanoma. The data is too preliminary to permit any conclusions.

F. Bladder Cancer. The DCT has no contract in bladder carcinoma. Due, however, to the high activity of cis-platinum in this tumor and the uniformly poor prognosis of patients with B and C lesions, the Division has agreed to sponsor, in part, a study by Bladder Collaborative Group A (supported by the Organ Site Program) a study of cis-platinum as an adjuvant to surgery and radiotherapy. This protocol is undergoing final institutional approval and will start entering patients shortly.

III. New Programs

A number of new areas are developing and are being considered for support within FY 77 and 78. The major determinant to developing these programs is the availability of new leads which are deemed worthy of exploitation. The following areas are at various stages of development and revision:

A. Bladder Cancer. Adjuvant studies in Stage D, after total cystectomy are being actively explored. The predictive pattern of relapse (up to 80% in one year) and availability of active agents (such as cis-platinum) make this a fertile area for future investigation.

B. Breast Cancer. New studies to evaluate the integration of endocrine and chemotherapy in this disease are planned via contract.

C. Colon Cancer. A new adjuvant program will begin within the summer of 1977 to test chemotherapy or immunotherapy in high risk-to-recurrence colon cancer, and chemotherapy or radiotherapy in rectal cancer. The National Surgical Adjuvant Breast Project has been awarded a contract to carry on this work in bowel cancer.

D. Head and Neck Cancer. An RFP has been issued, with deadline for receipt of proposals by September, 1977. Studies are planned to include: 1) Preoperative chemotherapy in oral cavity region. 2) Preoperative chemotherapy in laryngeal region, and 3) Chemotherapy prior to irradiation in the oral cavity region: impact on resectability, and evaluation of maintenance chemotherapy.

E. Lung Cancer. Three studies are expected to begin in resectable lung cancer beginning July, 1977, with six participating institutions: M.D. Anderson, Mayo, Vanderbilt, University of Toronto, UCLA, and Fred Hutchinson Cancer Center (Seattle). In addition, a pathology reference center will be funded (at M.D. Anderson Hospital).

F. Endometrial Cancer. Integration of endocrine therapy with chemotherapy is planned in this disease also, as receptor techniques improve.

G. Prostate Cancer. Patient resources of the VA Cancer Urological

Research Group may provide the opportunity to test chemotherapy on a wider scale; studies up till now being inconclusive because of small numbers.

H. Testicular Cancer. Recent advances in chemotherapy of this disease has led to formulation of a Stage II protocol which will be performed by cooperative groups and individual institutions under the coordination of NCI staff from the Branch.

I. Osteosarcoma. Support for a randomized adjuvant study in this disease versus no treatment has been instituted.

Other planned programs of the CMB include: - support for standardization and computerization of data from Phase II/III contractors, particularly in lung, breast, colon, sarcoma, and melanoma.

- expansion of Extramural Clinical Trials Office (ECTO) to be the operations office of these clinical contract groups such as testicular, GI Tumor Study Group, and the Lung Cancer Study Group.
- increased statistical input from intramural program in providing support for various contract supported trials.
- continued support for hyperalimentation programs and evaluation of nutritional effects of malignancy and treatment.
- evaluation of biological markers concurrent with clinical trials, with particular emphasis on lung and gastrointestinal cancers is planned in coordination with Frederick Cancer Research Center.

Expansion into all these areas will require additional staff members to function both as disease coordinators and modality consultants. Consolidation of the CMB with the CIB is expected to provide the expertise required to carry out these programs both within contract and grant supported groups.

IV. Peer-Review Committee Support.

The annual reports for the Peer-Review Technical Review Committees, Clinical Trials Committee and Combined Modality Committee, appear as Appendix 1 and 2.

The CMB staff members, Dr. Handelsman and Dr. McGuire, were Executive Secretaries of the Committees and responsible for convening the meetings and providing the appropriate materials for the reviewers. Reviews emanating from these committees provided additional directions to many of the CMB programs, as well as exposing CMB project officers to critical analysis and review of all the clinical trials contract projects. In new programs, the committees were particularly helpful not only in selecting the most appropriate respondents to RFP's, but also in providing the direction of the new therapeutic effort. Thus, the activities of the CMB are closely integrated with expert review process, in addition to the guidance received from NCI senior staff.

V. Publications

1. Handelsman, H.: The treatment of lung cancer; perspectives and critique. Journal of Surgical Oncology , 1977 (in press).

2. Handelsman, H.: The limitations of model systems in prostatic cancer. Oncology, 1977 (in press).

3. McGuire, W.P.: Cancer of the endometrium - A treatment strategy. In: Working Document on Cancer Treatment, U.S. Department of Health, Education, and Welfare, 1977 (in press).

4. McGuire, W.P. and Young, R.C.: Randomized clinical trials in ovarian cancer. In: Cancer Therapy: Prospective Randomized Clinical Trials. Editor, M. J. Staquet, Raven Press, 1977, (in press).

5. Myers, C.E., McGuire, W.P., Liss, R.H., Itrim, I., Grotzinger, K. and Young, R.C.: Adriamycin: The role of lipid peroxidation in cardiac toxicity and tumor response. Science, 1977 (in press).

6. McGuire, W.P., Young, R.C., Grotzinger, K., Myers, C.E.: Effects of tocopherol or adriamycin-induced cardiomyopathy and tumor response. Proc. Amer. Assoc. of Cancer Res., 1977 (in press).

7. Brereton, H.D., McGuire, W.P., Ihde, D., Levine, A.S., and Young, R.C.: Candida infections: Clinical and pathological correlations in 168 consecutive autopsy proven cases in cancer patients. Proc. Amer. Assoc. Cancer Res., 1977 (in press).

VI. Presentations

1. McGuire, W.P.: Chemotherapy of Breast Cancer and Lymphoma. Second Annual Oncology Conference of Peru. Lima, Peru, October, 25-29, 1976.

2. McGuire, W.P.: Future of Chemotherapy in the Treatment of Gynecologic Malignancies. Gynecologic Oncology Group Meeting, Miami, Florida, January 21, 1977.

3. McGuire, W.P.: Current clincial trials in lung cancer - An overview. U.S.- Japan Agreement on Lung Cancer. Los Angeles, California, March 1, 1977.

4. McGuire, W.P.: The Drug Development Program of the National Cancer Institute. Jackson Academy of Medicine. Jackson, Mississippi, March 28, 1977.

5. Adjuvant Chemotherapy, Adjuvant Radiotherapy, and the Role of Hormone Receptors in Breast Cancer. Medical Surgical Grand Rounds. Jackson, Mississippi, March 29, 1977.

6. Chemotherapy of Carcinoma of the Corpus Uteri and Uterine Cervix - A Review. Continuing Medical Education Seminar, Arlington Hospital, Arlington, Virginia, April 23, 1977.

VII. Committee Participation

Harry Handelsman, D.O.

Executive Secretary, Combined Modality Committee
Member, National Prostatic Cancer Project

William P. McGuire, M.D.

Executive Secretary, Clinical Trials Committee

Clinical Trials Contract Review Committee
National Institutes of Health
National Cancer Institute

Function:

The Clinical Trials Committee shall advise the Director, National Cancer Institute, and the Director, Division of Cancer Treatment concerning the scientific merit of proposals for contracts submitted in the area of clinical trials to the Division of Cancer Treatment. As experts in their fields, the committee members shall survey the status of cancer treatment and recommend areas of research to be supported by the Division of Cancer Treatment.

Dates and Places of Meetings:

1. December 9-10, 1976, Building 31, Conference Room 8, NIH, Bethesda, Maryland.

2. March 31, 1977, Building 31, Conference Room 8, NIH, Bethesda, Maryland.

Summary of Activities and Recommendations:

1. December 9-10, 1976:

An unsolicited proposal by Memorial Sloan-Kettering Cancer Center for use of high-dose MTX in pediatric brain tumors was discussed. Dr. Davis moved that the proposal did not justify sole source funding and their proposal should be referred to the Brain Tumor Study Group. The Committee unanimously passed the motion.

Dr. Ziegler discussed the history of the Uganda Cancer Center. Dr. Olweny was present from Kampala and reviewed past experience in the areas of Burkitt's lymphoma, Hodgkin's disease, Kaposi sarcoma and hepatocellular carcinoma. Four (4) options were entertained by the Committee: 1. Fund at requested $75,000.00 level, 2. Stop funding, 3. Decremental funding, and 4. Increase funding. Dr. Burchenal suggested funding $75,000 and an additional $75,000 if needed in the future. Dr. Lee moved that funding for $75,000 be approved and the progress reviewed in a year. The motion was unanimously adopted.

Dr. Arthur Levine, Chief, Pediatric Oncology and Project Officer for protected environment contracts, reviewed the rationale for the study. The Los Angeles Children's Hospital contract renewal and progress was reviewed. Several members made the point that accrual had dropped to 1/3 expected, costs per patient had risen to $25,000.00, CR and infection rates in both groups were equal and granulocyte counts in protected patients was about equal to controls although there was a 50% increase in amount of chemotherapy suggesting that doses were too low. Dr. Levine recommended one terminal year funding. Dr. Henry moved that funding be terminated now and the motion carried unanimously. The M.D. Anderson Hospital contract renewal was considered next. Members noted that accrual had been good with costs per patient at $12,000.00. A major deficit was lack of a clause for dose escalation in control patients. Dr. Levine recommended that funding continue for a terminal 18 months. Dr. Lee moved that funding continue at the requested level with the provision that only sarcoma and breast patients be studied and that the breast protocol be rewritten to provide for dose escalation in control patients and that there be stratification on basis of metastatic site. The motion was unanimously adopted.

Drs. Muggia and McGuire outlined the rationale for the new RFP in resectable lung cancer.

Seventeen proposals were evaluated from Albert Einstein, Roswell Park, UCLA, Bowman-Gray, Ohio State, Mayo Foundation, St. Vincent's Hospital of New York, Memorial Sloan-Kettering, University of Hawaii, Vanderbilt University, University Hospital of Boston, Harbor General Hospital, Columbia University, University of Toronto, Fred Hutchinson Cancer Center, M.D. Anderson Hospital, and University of Capetown. Nine were found technically acceptable and received the following priority ranking (1-9):

Mayo - 1.5
M.D. Anderson - 1.6
Fred Hutchinson - 3.5
Vanderbilt - 4.5
UCLA - 5.1
Toronto - 6.0
Memorial - 7.1
Ohio State - 7.2
Harbor General - 8.7

Contract negotiations resulted in funding the first six institutions.

2. March 31, 1977

The first item of discussion was an appeal by Dr. Bodey of M.D. Anderson Hospital regarding the Committee's previous decision to require drug escalation in breast cancer patients outside the laminar flow room in the same manner as those in protected environments. Dr. Levine reviewed the correspondence between NCI and M.D. Anderson which had transpired since the previous meeting. Dr. Bodey next spoke of his concern about dose escalation because of previous experience in AML with fatal infections following long periods of drug-induced bone marrow aplasia. After a lengthy discussion, the

Committee voted unanimously to both refund the anaerobic studies and to allow the Investigators at M.D. Anderson more freedom in determining advisability of dose escalation outside the PE so long as they did agree to escalate in absence of fever and only moderate leukopenia. They also agreed to allow re-entry of patients with non-Hodgkin's lymphoma during the next year.

Dr. Michael Walker reviewed the progress of the Brain Tumor Study Group. The dialogue which followed his presentation emphasized the need for the Group to simplify study design in Phase II studies to more rapidly screen new agents, collect data on cytotoxic effects of steroids, develop protocols for therapy of metastatic CNS lesions, develop protocols using newer radiotherapy techniques, tighten control of individual institutions to decrease case ineligibility and publish more. There was unanimous agreement to refund the Group for another year, but attention should be turned toward the items noted above.

The Contract with Hebrew University concerning use of MER in treatment of AML was reviewed by Dr. Poplack. The progress report by Dr. Weiss on basic immunologic data was felt to be sound, and all members agreed that support of this work should be continued. Since it has no direct bearing, however, on cancer treatment, it would best be funded through the Immunology Branch of the DCBD. Dr. Poplack will explore that possibility. The progress report for the clinical portion by Dr. Izak was felt to be poorly designed by all three reviewers. Since there are similar ongoing studies in the U.S., and there is no indication that there will be meaningful data forthcoming from this study, there was unanimous agreement that this Contract be terminated immediately.

Review of the Mt. Sinai Hospital Contract in advanced ovarian cancer was very positive. The Committee voted unanimously to refund the project at the level requested, and Dr. McGuire will represent the Contract to CTPS for additional funding within the next month.

The M.D. Anderson Hospital Contract for advanced ovarian cancer was reviewed next. The Committeee had only the semi-annual progress report to review and voted unanimously to withhold funding of this Contract until they had an opportunity to review the new Progress Report and Renewal Proposal. Dr. McGuire will mail these materials upon receipt.

The five Phase II/III Contracts were reviewed next. Sidney Farber Cancer Center was favorably reviewed and unanimously voted for continuation at requested level of funding. Memorial Sloan-Kettering also received a very favorable review. After some discussion concerning their request for 50% funding of a mini computer under this Contract, it was decided unanimously to approve the purchase. M.D. Anderson was reviewed and found acceptable from the point of numbers of patients put on study. The Committee voted unanimously to approve the Contract for another year, but to require studies with more rational design. Continuation of studies without proper controls or studies which are of such complexity that the data analysis is not possible, e.g., multiple drug combinations with immune therapy, will no longer be tolerated and will eventuate in Contract termination. Review of Mayo Clinic resulted in the Committee recommendation that this Contract be reviewed again in six months. Accrual has been significantly less than in other institutions and there are too many studies for the numbers of patients resulting in premature cancellation of some studies. Mayo must approximately double their accrual and limit the numbers of studies. Wayne State University was reviewed and again the Committee elected to re-examine the progress at six months. Accrual overall has been adequate, but the contribution from Henry Ford Hospital has been poor.

3. A progress report of the M.D. Anderson Hospital Ovarian Contract was mailed to all Committee members and this resulted in a decision to decrease funding to $100,000 and to re-examine the progress at six months.

APPENDIX 2, ANNUAL REPORT

Combined Modality Contract Review Committee
National Institutes of Health
National Cancer Institute

Function:

The Combined Modality Committee shall advise the Director, National Cancer Institute and the Director, Division of Cancer Treatment concerning the scientific merit or proposals for contracts submitted in the area of combined modality to the Division of Cancer Treatment. As experts in their fields, the committee members shall survey the status of cancer treatment and recommend areas of research that should be supported by the Division of Cancer Treatment.

Dates and Places of Meetings:

1. September 9, 1976, Building 31, NIH, Bethesda, Maryland
2. October 5, 1976, Building 31, NIH, Bethesda, Maryland
3. February 4, 1977, Building 31, NIH, Bethesda, Maryland
4. May 28, 1977, meeting cancelled, but mail review was held.

Summary of Activities and Recommendations:

1. September 9, 1976:

This meeting was held to consider the unsolicited proposal from the National Surgical Adjuvant Breast Project to conduct clinical trials in colorectal carcinoma.

The proposal was unanimously approved, with specific budgetary items to be negotiated by the National Cancer Institute Contract Officer.

2. October 5, 1976:

This meeting was scheduled to review the renewal proposals from the Gastrointestinal Tumor Study Group participants in clincal trials for patients with pancreatic and gastric cancers and renewal proposals for the current contractors conducting Phase I clinical trials. It was noted that patient accrual lagged behind expectations in most institutions, and budget will therefore be revised downward.

A. Pancreatic Contract Renewal Proposals

1. Yale University - NO1-CM-43797
   Renewal approved.
2. Mayo Foundation - NO1-CM-43796
   Renewal approved.

3. University of Miami - NO1-CM-43800
   Renewal approved.
4. Roswell Park Memorial Institute - NO1-CM-43794
   Renewal approved.
5. Sidney Farber Cancer Center - NO1-CM-43799
   Renewal approved.
6. University of California, Los Anglees - NO1-CM-43798
   Renewal approved.
7. University of Southern California - NO1-CM-43795
   Contract extended to March 31, 1977 without additional funds.

B. Gastric Contract Renewal Proposals

1. Mayo Foundation - NO1-CM-43783
   Renewal approved.
2. Roswell Park Memorial Institute - NO1-CM-43782
   Renewal approved.
3. Sidney Farber Cancer Center - NO1-CM-43781
   Renewal approved.

C. Phase I Contract Renewal Proposals

1. Mayo Foundation - NO1-CM-53838
   Renewal approved.
2. Mt. Sinai School of Medicine - NO1-CM-53837
   Renewal not approved.
3. Children's Hospital of Los Angeles - NO1-CM-43803
   Renewal approved.
4. University of Kansas - NO1-CM-43802
   Renewal approved.
5. Memorial Sloan-Kettering Cancer Center - NO1-CM-43804
   Renewal approved.
6. M.D. Anderson Hospital & Tumor Institute - NO1-CM-43801
   Renewal approved.

3. February 4, 1977:

This meeting was scheduled to review the renewal proposals from the Gastrointestinal Tumor Study Group participants in clinical trials for patients with colorectal carcinomas, renewal proposals for contractors conducting Phase I clinical trials, renewal proposals for Phase II trials in gastrointestinal cancer, renewal proposal for the NCI-Milan contract for clinical trials in breast, GI, and brain tumors, renewal proposal for the Georgetown University Gastrointestinal Research Program, and renewal proposals for Phase II/III trials in gastric and pancreatic carcinomas.

A. Colorectal Carcinoma Contract Renewal Proposals

1. Mayo Foundation - NO1-CM-57033
   Renewal approved.
2. University of California, Los Angeles - NO1-CM-67097
   Renewal approved.
3. Sidney Farber Cancer Center - NO1-CM-57035
   Renewal approved.
4. Roswell Park Memorial Institute - NO1-CM-57034
   Renewal approved.
5. Mt. Sinai School of Medicine - NO1-CM-67096
   Renewal approved.
6. Georgetown University - NO1-CM-67094
   Renewal approved.
7. Albany Medical College - NO1-CM-57032
   Renewal approved.
8. Memorial Sloan-Kettering Cancer Center - NO1-CM-53844
   Renewal approved.
9. University of Miami - NO1-CM-67093
   Renewal approved.
10. University of Chicago - NO1-CM-67095
    Renewal approved.

It was noted that accrual lagged behind expectations in most institutions; budgets will be revised downward.

B. Phase I Studies of New Anticancer Agents

1. Sidney Farber Cancer Center - NO1-CM-53837
   Renewal approved
2. Mt. Sinai School of Medicine - NO1-CM-53837
   Re-review of this contract after appeal of the previous recommendation of disapproval.
   Renewal approved.

C. Phase II Trials in Gastrointestinal Cancer

1. University of Leeds (England) - NO1-CM-43727
   Renewal approved.
2. Mayo Foundation - NO1-CM-02066
   Renewal approved.

D. Clincial Trials in Breast, GI, and Brain Tumors

1. NCI-Milan (Italy) - NO1-CM-33714
   Renewal approved.

E. Gastrointestinal Research Program

1. Georgetown University - NO1-CM-67110
   Renewal approved.

F. Phase II/III I Trials in Gastric Cancer

1. University of Southern California - NO1-CM-53842
   Renewal approved.

G. Phase II/III Trials in Pancreatic Carcinoma

1. University of Southern California - NO1-CM-43795
   Renewal not approved.

4. May 28, 1977:

A. Breast Cancer Contracts

1. Roswell Park Memorial Institute, with Albany Medical College subcontract. Review indicates very good performance and recommends extension until recompetition due.

2. Mayo Clinic
   Review indicates very good performance, and recommends extension until recompetition due.

INVESTIGATIONAL DRUG BRANCH

The Investigational Drug Branch (IDB) has the primary mission of sponsoring new investigational drugs for clinical trials and of evaluating them for antitumor efficacy. It does this by pursuing several objectives: (a) obtaining Investigational New Drug exemption (IND) authorization from the Food and Drug Administration (FDA), (b) managing the Phase I Working Group consisting of both contract supported and other unsupported members, (c) monitoring the clinical trials conducted by these investigators, (d) meeting FDA regulatory affairs responsibilities, and (e) regulating and distribution of investigational new drugs. It also has the objective of sponsoring and coordinating clinical pharmacology of new drugs and of managing a contract program for the biochemical and biological characterization of new drugs.

The permanent professional staff of the Branch includes one physician, one pharmacologist, one biochemist, and one pharmacist. Five staff associate physicians and one visiting fellow physician are professional staff who spend one year with the Branch.

The coordination of all the functions of the Investigational Drug Branch is done by the Office of the Chief. The regulatory function and distribution of investigational drugs throughout the United States and the world is provided by the Drug Liaison and Distribution Section, IDB.

Monitoring of clinical studies and chemotherapy evaluation.

Phase I studies: Ten investigational drugs sponsored by the DCT were studied in Phase I trials:

Chlorozotocin	NSC-178248
Gallium Nitrate	NSC-15200
Anguidine	NSC-141537
Aminothiadiazole	NSC-4728
Dichloroallyl lawsone	NSC-126771
Maytansine	NSC-153858
Neocarzinostatin	NSC-157365
Deazauridine	NSC-126849
Hycanthone	NSC-142982
Rubidazone	NSC-164011

The Phase I studies were conducted by members of the Phase I Working Group, which consists of clinical investigators from four branches of the NCI Intramural Program, clinical investigators from seven institutions awarded Phase I contracts and several institutions conducting Phase I studies without financial support.

Five Phase I drugs are entering Phase II studies. Pharmacokinetics studies in man were done on four Phase I drugs. The coordination of Phase I studies is accomplished by ad hoc telephone conferences and by Phase I Working Group Meetings approximately three time a year. Detailed information on the current activities of the Phase I Working Group Meeting is available from the Investigational Drug Branch.

Phase II Studies: The update and exchange of information on Phase II-III clinical studies is done by the New Drug Liaison Meetings. Minutes of these meetings are available from the Investigational Drug Branch.

Liaison with Cooperative Groups and Phase II Contractors: The members of the IDB professional staff attend cooperative group meetings. They actively participate also at the Phase II-III contractors meetings as well as in the disease oriented discussion and coordination meetings organized by the CTEP.

Feedback to Developmental Therapeutics Program: There has been a continuous feedback to the Developmental Therapeutics Program through several mechanisms (Operating Committee, Drug Evaluation Committee, and Decision Network Committee). In addition, critical analysis of several parts of the Program has been undertaken by the Investigational Drug Branch which is resulting in several studies such as correlation of clinical and experimental animal antitumor activity of investigational drugs, correlation of clinical and animal toxicity, setting up a running talley for clinical evaluation of Phase II-III clinical studies, critical evaluation of investigational anticancer drugs from foreign resources, etc.

DRUG LIAISON AND DISTRIBUTION SECTION

Food and Drug Administration Activities

Investigational New Drug Notices: There were five investigational new drug (IND) notices prepared and submitted to either the Bureau of Drugs or Bureau of Biologics, Food and Drug Administration (FDA).

These drugs are:

Chlorotozotocin	NSC-178248
Deazauridine	NSC-126849
RO-07-0582	NSC-261037
Levamisole	NSC-177023
Rubidazone	NSC-164011

Four other investigational drugs are being prepared by IND notices:

Bruceantin	NSC-165563
Pyrazoloimidazole	NSC-51143
AMSA	NSC-249992
Indicine-N-oxide	NSC-132319

Immunology Liaison: The Section provides a liaison between the Bureau of Biologics, Food and Drug Administration, and the clinical cooperative study group regarding regulatory requirements for cooperative study group trials which utilize immunotherapy.

Drug Distribution Activities: During the past year the Section continued the development of a clinical drug order verification procedure. Contract support from the Value Engineering Company was obtained, and a systems analysis of the drug distribution procedures was undertaken. The objective of this system is to insure that investigational anticancer drugs are distributed efficiently and in conformance with the Food and Drug Administration requirements.

CONTRACT ACTIVITIES

These contracts are designed to conduct Phase I clinical studies with new anticancer drugs sponsored by the Division of Cancer Treatment, National Cancer Institute or to conduct Phase I clinical studies with new combinations or regimens mutually agreed upon.

CHILDREN'S HOSPITAL OF LOS ANGELES (NO1-CM-43803). Phase I studies have been conducted on dianhydrogalactitol (NSC-132313), β-deoxythioguanosine (NSC-71851), and neocarzinostatin (NSC-157365) during the past year.

MAYO FOUNDATION (NO1-CM-53838). Phase I studies on maytansine (NSC-153858), hycanthone (NSC-142982), rubidazone (NSC-164011), and chlorozotocin (NSC-178248) have been conducted during the past year.

MOUNT SINAI SCHOOL OF MEDICINE (NO1-CM-53837). Phase I studies on vindesine (NSC-245647), pyrazofurin (NSC-143095), and neocarzinostatin (NSC-157365) have been conducted in the past year.

M. D. ANDERSON HOSPITAL AND TUMOR INSTITUTE (NO1-CM-43801). Phase I studies have been conducted on anguidine (NSC-141537), neocarzinostatin (NSC-157365), maytansine (NSC-153858), deazauridine (NSC-126849), gallium nitrate (NSC-15200), hycanthone (NSC-142982), and dichloroallyl lawsone (NSC-126771) during the past year.

MEMORIAL HOSPITAL FOR CANCER AND ALLIED DISEASE (NO1-CM-43804). Phase I studies on thiadiazole (NSC-4728), vindesine (NSC-245467), tetrahydrouridine and cytosine arabinoside, and chlorozotocin (NSC-178248) have been conducted during the past year.

SIDNEY FARBER CANCER CENTER (NO1-CM-53839). Phase I studies on maytansine (NSC-153858), neocarzinostatin (NSC-157365), high dose methotrexate-thymidine rescue and aminopterine-citrovorum factor rescue have been conducted in the past year.

UNIVERSITY OF KANSAS MEDICAL CENTER (NO1-CM-43802). Phase I studies have been conducted on dianhydrogalactitol (NSC-132313), anguidine (NSC-141537), and chlorozotocin (NSC-178248) during the past year.

The following contracts are for the biochemical and molecular biological evaluation of investigational new drugs being developed by the DCT Program.

SOUTHERN RESEARCH INSTITUTE (NO1-CM-43784). The work scope of this contract has been subdivided into five project objectives: (1) To determine if new antitumor drugs have biochemical mechanisms of action similar to those of clinically established antitumor drugs. (2) To identify or provide clear leads to the biologically significant biochemical properties of new antitumor drugs. (3) To identify and study the molecular mechanism of action of new antitumor drugs. (4) To select from among active structural analogs of clinically evaluated antitumor drugs, those which may have more desirable biochemical properties. (5) To develop and evaluate biochemical rationales for the combined use of clinically established antitumor drugs. This contractor has published several papers resulting from this contract, and is conducting outstanding investigations. Information gathered from this effort has been very useful in preparing Biochemical Data Sheets. This is one of the most productive groups in the biochemical research area.

M.D. ANDERSON HOSPITAL AND TUMOR INSTITUTE (NO1-CM-43786). The objective of this project is to provide a detailed study of selected drugs in development for clinical trials on polynucleotide metabolism. Dr. Hurlbert reports that Cain's acridine (NSC-249992) produces extensive fragmentation of cellular DNA. Alanosine, which has previously been found to inhibit adenylosuccinate synthetase, does not have a direct effect on the enzyme. It is metabolized to small molecular weight inhibitor which is inactive in vitro. The structure of this metabolite has not yet been identified but is thought to be the alanosine analog of N-succinylcarboxamide aminoimidazole ribotide.

ALBERT EINSTEIN COLLEGE OF MEDICINE (NO1-CM-43787). The major emphasis of this project is to provide a detailed evaluation of new antitumor drugs which affect protein synthesis. Studies with bruceantin and anguidine have been completed and published. Both these drugs are proceeding towards Phase I studies. In depth studies are being conducted with VP-16 (NSC-141540). VP-16 does not bind to tubulin. It degrades cellular DNA. Vincristine (NSC-67574) binds to tubulin but does not degrade cellular DNA. A compound combining both these activities is under investigation.

BRIGHAM YOUNG UNIVERSITY (NO1-CM-43790). The objective of this project is to provide detailed information of the effects of selected antitumor drugs on folate metabolism. Approximately 30 antitumor agents, most of which are folic acid or antifol triazine derivatives have been tested as inhibitors of enzymes of folic acid metabolism.

CLINICA PUERTA DE HIERRO (NO1-CM-53792). The initial intended objective of this contract was to study the details of the effects of anticancer agents on electron transport processes. Approximately 40 anticancer agents were evaluated for their potency as inhibitors of oxygen utilization in isolated mitochondria and in intact Ehrlich Ascites cell. Currently, Dr. Gosalvez is investigating the effect of adriamycin on ion transport across membranes and its effect on antigen capping. Preliminary evidence indicates that ICRF-159 (NSC-129943) is also active in this system. Further evaluation is being done.

UNIVERSITY OF MINNESOTA (NO1-CM-43788). The major emphasis of this project is directed toward determining the cell uptake transport processes of selected antitumor drugs. In addition, the effects of membrane active, natural substances on the uptake and effectiveness of antitumor drugs are being evaluated. Some information on the transport properties of nine antitumor agents have been reported.

MICHIGAN CANCER FOUNDATION (NO1-CM-53789). The objective of this project is to provide detailed information on the effects of antitumor agents on membrane properties and structure. Compounds supplied for testing are evaluated in the following systems:
(1) Reversible and irreversible effects on uptake of uridine and cycloleucine by L5178Y and L1210 cells, and actinomycin D by L1210,
(2) Effects on partitioning behavior in systems A (Phosphate-Dextran-PEG) and B (NaCl-Dextran-PEG) of L1210 and L5178Y cells,
(3) Effects on microvilli as seen under scanning EM.

NSC-142982 (Hycanthone) a DNA intercalator with mutagenic action in L5178Y cells inhibited uptake of uridine in L1210 and L5178Y cells. The drug markedly altered partitioning behavior, increasing the coefficient in system A (indicating enhanced electronegativity of cells) but slightly decreased the coefficient in system B.

Recently a study of hematoporphyrin (NSC-59265) indicated a profound effect on nucleoside uptake, actinomycin D uptake, and alteration of cells surface hydrophobicity. Several analogs were also signaled for equal activity. These analogs have been sent to Dr. P. Meeker at A.D. Little for *in vivo* evaluation.

UNIVERSITY OF PENNSYLVANIA (NO1-CM-53830). The general objective of this project is to evaluate the effects of selected antitumor agents on the bioenergetic performance status of intact organs. The specific research has been directed toward determining the mechanism of adriamycin cardiotoxicity. More recently Dr. Chance has observed that adriamycin stimulates the formation of superoxide anion radical in mitochondria *in vitro*, raising the possibility of secondary damage due to this species.

UNIVERSITY OF CALIFORNIA (NO1-CM-43791). The objective of this project is to provide biochemical rationales for the combined use of radiotherapy and clinically established antitumor drugs. This is being approached by studies on cell survival in cell culture and on DNA damage as measured by sedimentation techniques.

HARVARD UNIVERSITY (NO1-CM-53825). The objective of this project is to evaluate the role of carbohydrate metabolism in the development potential of chemotherapeutic agents.

It was found that the nucleoside activation of glucose and mannose were sensitively dependent on the prior carbohydrate nutrient history of the cell line (eg. glucose, mannose, or sugar deprivation). At concentrations having no effect on cell growth or RNA or protein synthesis, 6-thioguanine and 6-thioguanosine significantly inhibited the conversion of glucose and to UDP-glucuronic acid.

In vivo, mannose, glucose, and galactose all failed to increase the survival of P388 leukemia bearing mice. However combinations of deoxyglucose and 6-thioguanine did increase the mean survival time and produced long term survivors. This effect appears to be a synergistic one. These studies were expanded to include deoxygalactose.

NEW YORK STATE DEPARTMENT OF HEALTH (NO1-CM-43785). The "Profile of Drug Interaction" system under development is designed to (a) determine the similarity of new anticancer agents to agents of known mechanism of action, (b) to determine whether or not all the agents in a series of structural analogs are also biochemical analogs.

The patterns generated to date indicate that 17 of the 34 drugs being studied have unique interaction patterns. A group of DNA reactive drugs also appears to be characterized by an additive interaction effect with most of the other drugs. It has been noted that Anguidine has an antagonist interaction effect with many of the other drugs in the panel. On the basis of this observation the effects of anguidine in combination with several of these chemotherapeutic agents on experimental tumor system are being evaluated by the Drug Evaluation Branch.

BIOTECH RESEARCH LAB., INC. (NO1-CM-67052). The objective of the project whose progress is reported here is to set up and apply an *in vitro* primary screening system in which compounds are tested for their possible value as chemotherapeutic agents against human neoplasms by testing their effect on the functioning of oncogenic viruses. The screening system being used in the project screens for compounds which selectively inhibit the following: (1) transformation of cells by a DNA or RNA oncogenic virus, (2) growth of transformed cells relative to the untransformed counterpart, (3) expression of an antigen associated with infection and transformation by a DNA or RNA virus, (4) activity of purified RNA-dependent DNA polymerase.

Thirty-nine drugs have been subjected to this screen. Four have been shown to be active in the RNA oncogenic virus system (NSC-248557), Thioguinoxaline; NSC-84423, Phenanthridinium; NSC-163039, Virazole; NSC-195183, Thymidine polymer) while four others are active against the DNA virus system (NSC-245465, Paloxy polymer, NSC-172112, Spirohydantion mustard; NSC-143095 Pyrazomycin; NSC-123127, Adriamycin). Five of these compounds (NSC-172112, NSC-143095, NSC-140377, NSC-191959, and NSC-123127) also inhibited growth of the transformed line. Enzymatic inhibition was noted in five compounds, but did translate to inhibition of cell growth.

WASHINGTON UNIVERSITY (NO1-CM-43731). This project has as its objective the study of the effects of combinations of antitumor agents on normal and malignant cell populations in the mouse. Quantitative cellular assays are used and particular attention has been placed on the role of drug sequence and drug interval. The agents chosen for study (based on activity in human breast cancer) were adriamycin, cytoxan, 5-FU, methotrexate, L-PAM, vincristine, and x-ray.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 06600-02 ID

PERIOD COVERED

July 1, 1976 - September 30, 1977

TITLE OF PROJECT (80 characters or less)

Clinical Cardiotoxicity of Anticancer Agents

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

Daniel D. Von Hoff, M.D., Staff Associate, Investigational Drug Branch, Cancer Therapy Evaluation Program, DCT, NCI

Maxwell Layard, Ph.D., Biostatistician, Biometry Branch, Division of Cause and Prevention, NCI

Franco M. Muggia, M.D., F.A.C.P., Associate Director, Cancer Therapy Evaluation Program, DCT, NCI

COOPERATING UNITS (if any)

Biometry Branch, Division of Cancer Cause and Prevention
Division of Computer Resources and Technology

LAB/BRANCH

Investigational Drug Branch, Cancer Therapy Evaluation Program, DCT

SECTION

INSTITUTE AND LOCATION

National Cancer Institute, Bethesda, Maryland

TOTAL MANYEARS: 2.0	PROFESSIONAL: 1.5	OTHER: 0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This study was initiated to determine the effect of anticancer drugs on the heart in man. This is a major problem because it frequently limits the amount of an effective anticancer drug that can be given to a patient.

The research will determine (1) which drugs are cardiotoxic in man,
(2) relationship between total doses of drug and cardiotoxicity,
(3) the signs and symptoms of drug-induced cardiotoxicity,
(4) safest doses and schedules drugs to be used in patients.

In addition, the research should provide clues for designing newer, less cardiotoxic anticancer compounds.

PROJECT DESCRIPTION:

In the research period last reported (July 1, 1975 to June 30,1976) this research project studied one of the cardiotoxic anticancer agents, daunomycin. The study was reported in the American Journal of Medicine, 62: 200-208, 1977. The major finding in that study was that the drug was less cardiotoxic than previously appreciated. This finding has enabled the treating physician to give the drug over a larger period of time and thus give the patient a better chance of responding to therapy.

The second drug presently under investigation is adriamycin, a compound with good antitumor effect in breast cancer, sarcomas, gastric cancer, Hodgkin's disease and leukemia. This compound is also cardiotoxic. The present work is reviewing the clinical charts of 5,000 patients who have received the drug so we can define what factors make patients more susceptible to the drug-induced cardiotoxicity. If these can be defined, one can attempt to recognize these patients before treatment and monitor or hopefully prevent the devastating cardiac toxicity.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 06601-02 ID

PERIOD COVERED
July 1, 1976 - September 30, 1977

TITLE OF PROJECT (80 characters or less)
Correlation of Clinical and Antitumor Activity of Single Anticancer Drugs

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

Marcel Rozencweig, M.D., Visiting Fellow, Investigational Drug Branch, CTEP, DCT, NCI

Franco Muggia, M.D., F.A.C.P., Associate Director, Cancer Therapy Evaluation Program, DCT, NCI

John M. Venditti, Chief, Drug Evaluation Branch, DTP, DCT, NCI

COOPERATING UNITS (if any)
Drug Evaluation Branch, DTP, DCT, NCI

LAB/BRANCH
Investigational Drug Branch

SECTION

INSTITUTE AND LOCATION
National Cancer Institute, Bethesda, Maryland

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2	1.5	.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

It is the long range purpose of this project to study the correlation of the activity men and experimental animals of single anticancer drugs.

The ultimate goal of this project will be: (1) to define a predictive mathematical model of clinical activity of new anticancer drugs based on animal data; (2) to evaluate the predictive value in man of the present and the future animal tumor models for clinical studies.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION:

Sixty anticancer drugs have been introduced in clinical studies so far. The activity of all these drugs has been tested in five mice tumor systems i.e., P388 leukemia, L1210 leukemia (solid and ascites form), B16 melanoma, and Lewis lung carcinoma. For all these drugs, in each of these experimental systems, an ILS has been determined (ILS = Increase in life span of the treated mice as compared to the survival of untreated controls). The clinical activity of these drugs is presently evaluated by various tumor types.

For each of the most frequently investigated human tumors, a mathematical model will be defined according to the clinical and animal data. Hopefully, this model will allow us to predict the activity of investigational drugs in the most frequent tumor types based on ILS found in the previously mentioned experimental tumors. This model will also allow to determine the predictive contribution of new experimental tumors.

A continuous updating of the activity of investigational drugs will take place through a running talley system. This update will permit a permanent control and improvement of the model.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 06603-02 ID

PERIOD COVERED

July 1, 1976 - September 30, 1977

TITLE OF PROJECT (80 characters or less)

Correlation of Clinical and Experimental Animal Toxicity of Anticancer Drugs

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

Marcel Rozencweig, M.D., Visiting Fellow, Investigational Drug Branch, CTEP, DCT, NCI

Franco Muggia, M.D., F.A.C.P., Associate Director, Cancer Therapy Evaluation Program, DCT, NCI

A.M. Guarino, Ph.D., Chief, Laboratory of Toxicology, DTP, DCT,NCI

C.L. Litterst, Ph.D., Senior Staff Fellow, LT, DTP, DCT, NCI

Ira Kline, Ph.D., Expert Consultant, International Treatment Research, DCT, NCI

COOPERATING UNITS (if any)

Laboratory of Toxicology, International Treatment Research, DCT, NCI

LAB/BRANCH

Investigational Drug Branch

SECTION

INSTITUTE AND LOCATION

National Cancer Institute, Bethesda, Maryland

TOTAL MANYEARS: 2	PROFESSIONAL: 1.5	OTHER: .5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

It is the long range purpose of this project to study the correlation of the toxicity in men and experimental animals of single anticancer drugs.

The ultimate goals of this project will be: (1) to define a safe and effective starting dose of anticancer drugs when studied the first time in man; (2) to evaluate the predictive value of animal toxicity data in man.

PROJECT DESCRIPTION:

All the drugs which have been tested I.V. in men with the following schedules will be analyzed: high intermittent dose weekly and daily x 5. Clinical toxicity will be evaluated for all of these drugs.

Values of LD10, LD50, and LD90 will be determined at each of these schedules in rodents. Thorough anatomic examinations will also be performed at each of these lethal dose levels. A mathematical model will be tentatively defined for the correlation of the clinical and animal data. Hopefully, this model will enable to predict a safe but also an effective dose of investigational drugs in men based on toxicity data in rodents. This study should also show to what extent qualitative toxicology in rodents might be predictive in men.

A continuous updating of the toxicity of investigational drug will take place through a running talley system. This update will permit a permanent control and improvement of that correlation.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 03579-04 LMCB

PERIOD COVERED

July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Effect of Chemotherapeutic Agents on L1210 Cell Cycle Progression

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Vincent H. Bono, Jr.	Chief, Investigational Drug Branch CTEP, DCT, NCI
	Robert L. Dion	Chemist, Laboratory of Medicinal Chemistry and Biology, DTP,DCT,NCI

COOPERATING UNITS (if any)

The Upjohn Company, Kalamazoo, Michigan, Cancer Research Contract NO1-CM-43743, Laboratory of Molecular Biology, Division of Cancer Biology and Diagnosis, NCI

LAB/BRANCH

Laboratory of Medicinal Chemistry and Biology

SECTION

Molecular Biology and Methods Development Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland

TOTAL MANYEARS: 1 1/4	PROFESSIONAL: 1 1/4	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Project is designed to investigate the effects of cancer chemotherapeutic agents on cell cycle progression based on their cytotoxicity in an in vitro L1210 murine leukemia system. DNA content distribution (DCD) curves are generated with the use of a flow microfluorometer and drug treated curves are compared with steady state DCD curves after analysis by computer. An attempt to classify these agents as to their mode of action (anti-metabolite, etc.) by computer analysis is in progress.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION:

This project is concerned with the development of techniques for the analysis of drug effects on the progress of cells through the proliferative cell cycle and their application to (a) the characterization of therapeutically active drugs and (b) the identification and possible selection of agents with potential therapeutic activity. The major methods employed in this project are (a) mammalian cell culture techniques applied to the growth of L1210 murine leukemia cells in suspension culture, (b) flow microfluorometric determination of cell DNA content using fluorescent hypotonic citrate/propidium iodide stained cells in a Cytofluorograf (Biophysics Systems, Inc.) interfaced with a multichannel pulse height analyzer and (c) processing, analyzing and plotting cell DNA content distributions (DCD) profiles by computer.

During the current year, 16 drugs of interest were evaluated for their effects on L1210 cell cycle progression: Cain's Acridine (NSC-141549), Tritylcysteine (NSC-83265), Ellipticine (NSC-71795), Coralyne (NSC-154891), Inox (NSC-118994), Adenox (NSC-266754D), Hycanthone (NSC-142982), "Tetrakis" (NSC-185954D), deoxyuridine-5-thiocyanate (NSC-159861), deoxyuridine-5-propynyloxy (NSC-267459), lapachol (NSC-11905), dichlorolapachol (NSC-126771), "Michigan protein" (NSC-174951), α-picolinic acid, Bouvardia ternifolia (NSC-227262), and Chartreusin (NSC-5159).

MAJOR FINDINGS:

Inox and Adenox at ED_{50} dose levels appear to block cell progression primarily in G_2 phase. There is a question of whether endoreduplication occurs as results varied according to the staining techniques employed.

"Michigan protein" was analytically separated by gel permeation chromatography in a 20% DMSO/neutral buffer system into a high molecular weight inactive species and a toxic moiety with a molecular weight of 1000-1500. The latter species comprises 1-2% of the bulk material. A single attempt at large scale purification of the active moiety was unsuccessful.

In a collaborative study with Drs. George Johnson and Alberto Fernandez-Pol, α-picolinic acid effects on DNA content distribution curves of normal and transformed rat kidney cells were investigated. Normal cells were reversibly arrested in G_1 phase whereas four lines of virally transformed cells showed responses which were dependent on the type of transforming virus. Kirsten sarcoma virus transformed cells are blocked in G_1. SV40 transformed cells progress to a G_2 block. Cells transformed by polyoma or Harvey sarcoma virus with Moloney virus coat have DCD profiles which indicate blocks in both G_1 or G_2. Cells transformed with Moloney sarcoma virus are not blocked in a specific phase of the cell cycle.

An additional collaborative study on L1210 survival and cell cycle progression was conducted with Dr. Bijoy Bhuyan of the Upjohn Company using the drug Chartreusin (NSC-5159) which binds to DNA. Results showed that in cells in S phase were more sensitive to the drug's toxic effect than cells in other phases of the cycle. Flow microfluorometry (FMF) revealed that effects on cell cycle progression were concentration and exposure time dependent. The DNA content distribution (DCD) curves for cells given a four hour exposure to drug followed by a subsequent four hour recovery in the absence of drug were remarkably similar to continual drug exposure for eight hours. With a 20 hour recovery, DCD curves reverted to essentially untreated control cuves whereas continuous exposure resulted in extensive degradation of cellular DNA.

This project relates to the Drug Developmental Program in providing characterization of the cell biological properties of therapeutically active drugs and in exploring the possibility of developing a new screen for the selection of drugs having therapeutic activity. In addition to continuing the current research effort, it is planned to attempt to develop a method whereby DNA content and synthesis rate of individual cells can be measured simultaneously.

Keywords: Cancer chemotherapeutic drugs, cell cycle progression, cell DNA content, cytotoxicity, endoreduplication, flow microfluorometry, hypotonic propidium iodide.

CONTRACTOR PUBLICATIONS:

1. Amos, H., Leventhal, M., Chu, L., and Karnovsky, M.: Modifications of mammalian cell surfaces induced by sugars: scanning electron microscopy. Cell. 7: 59, 1976.

2. Bennett, L. Lee and Allan, Paula, W.: Metabolism and metabolic effects of 8-Azainosine and 8-Azaadenosine. Cancer Res., 36: 3917-3923, 1976.

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